Review Article

Chemical Constituents and Biological Activities of Genus *Picrorhiza*: An Update

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Prakash *et al.*: Chemical Constituents and Biological Activities of Genus *Picrorhiza*

This review article on *Picrorhiza* genus comprehensively summarized the characterization, morphology, distribution, traditional uses, chemical constituents and biological activities of isolated individual constituents as well as plant extracts. It belongs to the Scrophulariaceae family, a small genera with two species, which are mainly found in Western and Eastern Himalayas at an altitude of 3000-5200 m. It has been used traditionally to cure many diseases like asthma, arthritis, cancer, diabetes, diarrhea, dyspepsia, fever, gastrointestinal problems, jaundice, urinary disorders. Its phytochemical analysis reported the presence of glycosides, aromatic esters, bis-iridoid, phenyl propenoids, alcoholic compounds and fatty acids. Chemical constituents of the genus *Picrorhiza* and its crude extracts with various solvents exhibit biological activities such as antimicrobial, anticancer, antiasthmatic, antidiabetic effects.

Key words: *Picrorhiza*, glycosides, antimicrobial, anticancer, phytochemicals

Plants and their parts have been used for medicinal uses since immemorial time. The first evidence marked on clay tablets in the cuneiform script is from Mesopotamia 2600 BC where the recipes of drug preparation have been written[1-3]. It is a universally accepted principle that human beings and diseases are born simultaneously. Vedas[4], Quran[5-7], Buddhism[8] and Bible[9] are the religious texts that have described diseases, drugs, and therapies. The entire knowledge of *Ayurveda* is derived from *Vedic* literature. *Vedic* system of medicine is the root of *Ayurveda* and it is supposed to be the science of all aspects of life[10]. Ancient philosophy deliberates the "secret of life and beyond" for health and medicinal plants[11]. No plant of this earth is useless from a medicinal point of view[12] .

The Himalayas, a mountain range in Asia covering mainly India, Pakistan, China, Tibet, and Nepal is known as a treasure house of the medicinal plants with around 8000 species in which 1748 species are known to have medicinal value[13-16]. A majority of the rural population (~90 %) depends upon the medicinal plants as a source of remedies[17-21]. Alone in India about 2000 tons of herb consumed annually[22-26]. Globally, the excessive use of synthetic drugs and their side effects have attracted the researchers towards herbals medicine in past years. Plants contain many different phytochemical compounds that are individually effective enough to treat chronic and infectious diseases[27,28].

The genus *Picrorhiza* is well known for its medicinal values since the ancient as well as modern period. There are not many reviews on *Picrorhiza,* except a few such as cellular differentiation, regeneration and secondary metabolite production in medicinal *Picrorhiza* species[29], reviews of its chemical constituents[30], phytopharmacological review on genus *Picrorhiza*[31], *Picrorhiza kurroa*: an ethnopharmacologically important plant species of the Himalayan region[22]. Therefore, there is a need to prepare an updated detailed review on the genus *Picrorhiza.* This review mainly deals with the isolation of plant extracts and individual chemical constituents of *Picrorhiza* and studies of biological activities shown by an individual chemical constituent as well as on solvent extracts carried out so far in the last decade, based upon the literature survey through PubMed, Mendeley, Ex Prisma.

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Classification:

Picrorhiza is commonly called *karu*[32], while scientifically it is placed in Kingdom: Plantae; Class: Dicotiledonal; Order: Scrophulariales; Family: Scrophulariaceae; Genus: *Picrorhiza*; Species: *kurroa, scrophulariiflora*; Botanical name: *P. kurroa* Royal ex Benth and *P. scrophulariiflora* Pennel[31].

Morphology:

The genera *Picrorhiza* is derived from 'picros' and 'rhiza', which means bitter root[33-35]. Genus *Picrorhiza* has two species namely *Picrorhiza kurroa* and *Picrorhiza scrophulariiflora*[36]. *P. kurroa* is smaller in height (5-9 cm) with smaller leaves (7-11 cm). The leaves of the plant are flat, oval, saw-toothed, five narrowed to a winged stalk, and sharply serrated. The flowers are white or pale purple and borne on a tall spike and its corolla is much smaller (0.8 cm), five-lobed to the middle, and with very much longer stamens. The rhizome is 2.5-12 cm long, sub-cylindrical, straight, or to some extent curved, and the external surface is coarse[37-40].

P. scrophulariiflora is longer than *P. kurroa* in height (8-12 cm) with longer leaves. The leaves of the plant are flat, oval, saw-toothed and its leaf blades are 10-14 cm in length. The flowers are dark blue-purple in color arising from a rosette of conspicuously serrated leaves and its corolla is 1.5 cm. Rhizomes are 2.5-12 cm long, sub-cylindrical, straight, or to somewhat curved and the rhizomes are evanescent inside[37].

Distribution:

P. kurroa occurs mainly in the Western Himalayas at an altitude of 3000-5000 m[34,41-44], while *P. scrophulariiflora* is found mainly in the Eastern Himalayas at an altitude of 4300-5200 m. *P. scrophulariiflora* species of this genus is found only in Nepal[45,46].

Traditional uses:

The World Health Organization (WHO) defined that the traditional medicine is a total sum of all knowledge and practices, which is explainable or not and it is used in diagnosis, prevention and elimination of physical, mental, or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation, whether verbally or in writing[46]. Traditional knowledge is passing from generation to generation without the aid of any documentation or keeping written records. This knowledge will be lost with succeeding generations if it is not rapidly researched and recorded[18]. Therefore, its documentation is of paramount importance and urgent so that it can be preserved and conserved[47,48]. Ethnobotanical surveys are effective methods in documenting and identifying medicinal plants used in the traditional knowledge system[49]. Both the species *P. kurroa* and *P. scrophulariiflora* have been used traditionally for the treatment of a large number of diseases long before the time[50,51]. The different parts of *P. kurroa* have been used to cure various diseases. The rhizomes of *P. kurroa* are used to cure asthma, arthritis, cancer, diabetes, dyspepsia, fever, gastrointestinal problems, jaundice, leukoderma, piles, snake bite, and urinary disorders.

The seeds of *P. kurroa* are used to treat diarrhea, fever, liver, and upper respiratory tract disorders, its leaves are used to cure diseases such as bilious fever and chronic infections, and its flowers are also used for the treatment of fever and blood purification[41,42,52-77].

Traditionally, stems of *P. scrophulariiflora* have been used to cure arthritis, asthma, choleretic, jaundice, liver disorder while its roots are useful for the treatment of bacterial infections, fever, jaundice, liver disorder, and inflammatory diseases[78-81]. *P. scrophulariiflora* is also used in traditional Chinese medicine for treating dysentery, jaundice and rheumatism[82].

Isolation of chemical constituents:

During the said period of review around 53 compounds have been isolated from these 2 species of genus *Picrorhiza*, out of which 38 are glycosides, 6 aromatic esters, 4 bis-iridoids, 4 phenylpropanoids and an alcoholic compound. From *P. kurroa,* a total of 36 compounds have been isolated, which included 26 glycosides, 6 aromatic esters and 4 bis-iridoids from different parts of the plants whereas 17 compounds have been isolated from *P. scrophulariiflora*, which contained only 12 glycosides, 4 phenylpropenoids and an alcoholic compound (Table 1). The structures of these compounds are given in fig. 1 (excluding compounds reported by Sah and Varshney[30]).

Glycosides are formed from the combination of a hydroxyl group of a simple sugar with another compound. From the genus *Picrorhiza,* 8 types of glycosides have been isolated, which included iridoid glycosides (23), aromatic aldehyde glycoside (1), aromatic aldehyde diglycoside (1), diphenolic tetra glycoside (1), phenolic glycosides (5), phenylpropanoid glycoside (2), secoiridoid glycosides (2) and hydroquinone glycosides (3) from different parts of the

TABLE 1: CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF GENUS *PICRORHIZA*

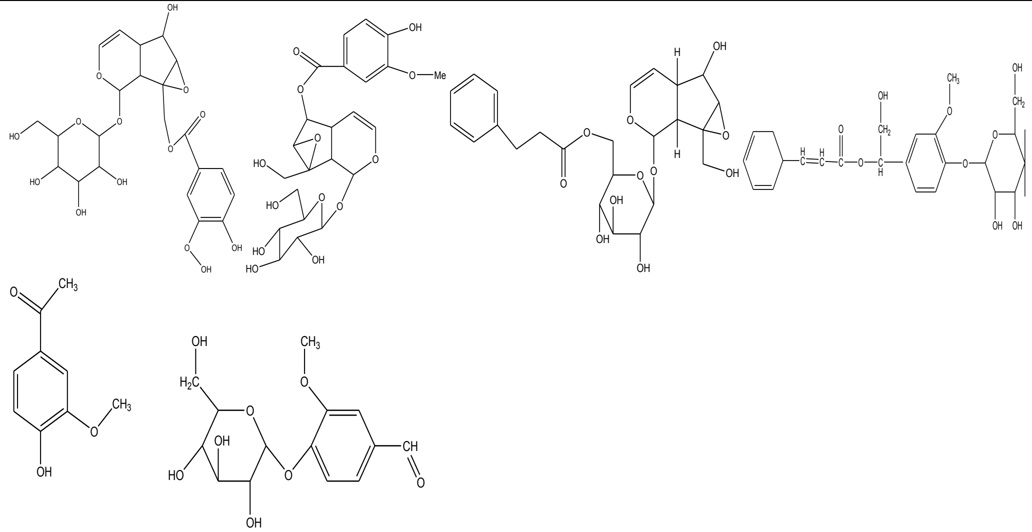
S. No.	Chemical constituents (No.)	Plant species	Plant Part	Solvent employed	Biological activities	Reference
			Glycosides			
1.	[6-[(1a,1b,2,5a,6,6a)-6-hydroxy-1a-(hydroxymethyl)-2,5a,6,6a-tetrahydro-1bHoxireno[5,6]cyclopenta [1,3-c]pyran-2-yl]oxy]-3,4,5-trihydroxyoxan-2-yl] methyl-3 phenylprop-2-enoat. (**1**) IG	P.K.	Rhizomes; Rhizomes and leaves	Methanol; Methanol: water (1:1); Ethanol and Water	Anticancer activity; Antiarthritis activity; Antidiabetic activity; Hepatoprotective activity.	[75,84,86,88, 89,90,92]
2.	[2-(hydroxymethyl)-10-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,9-dioxatricyclo[4.4.0.0^{2,4}]dec-7-en-5-yl] 4-hydroxy-3-methoxybenzoate. (**2**) IG	P.K.	Rhizomes; Rhizomes and leaves	Methanol; Methanol: water; Methanol and Water; Ethanol and Water	Antiarthritis activity; Antidiabetic activity; Hepatoprotective activity.	[75,84,88, 89,90,92]
3.	[5-hydroxy-10-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-3,9-dioxatricyclo[4.4.0.0^{2,4}]dec-7-en-2-yl] methyl 4-hydroxy-3-methoxybenzoate. (**3**) IG	P.K.	Rhizomes	Ethanol; Methanol	Antimicrobial activity; Anticancer activity.	[85,86]
4.	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3-methoxy-4-[3-phenylprop-2-enoyl] oxybenzoatedihydrate. (**4**) IG	P.K.	Rhizomes	Water; Methanol	Antidiabetic activity; Antimicrobial activity	[86,87]
5.	1-(4-hydroxy-3-methoxyphenyl)ethanone. (**5**) IG	P.K.	Rhizomes	Ethanol and Water	Antidiabetic activity.	[90]
6.	Vanillin-α-D-glucopyranoside. (**6**) AAG	P.K.	Rhizomes	Methanol	------	[91]
7.	Picraldehyde 4-O-α-D-glucopyranosyl-(6′→1″)- O-α-D-glucopyranoside. (**7**) AADG	P.K.	Rhizomes	Methanol	------	[91]
8.	Picrorhizaoside-A. **(8)** IG					
9.	Picrorhizaoside-B. **(9)** IG					
10.	Picrorhizaoside-C. **(10)** IG					
11.	Picrorhizaoside-D. (**11**) IG	P.K.	Rhizomes	Methanol	Hyaluronidase inhibitory activity	[110]
12.	Picrorhizaoside-E. **(12)** IG					
13.	Picrorhizaoside-F. **(13)** IG					
14.	Picrorhizaoside-G. **(14)** IG					
15.	3-methoxy-4-hydroxyphenyl-*n*-butanyl-α-O-D-glucopyranosyl-(6a→1b)- α-O-D-glucopyranosyl-(6b→1c)- α-O-D-glucopyranosyl-(6c→1d)- α-O-D-glucopyranosyl-4d-3′-methoxy-4′-hydroxyphenyl-*n*-pent-7′,9′-dien-11′-oate. (**15**) DPTG	P.K.	Rhizomes	Methanol	------	[91]
16.	3-phenylprop-2-enoic acid. (**16**) IG					
17.	3-(4-hydroxy-3-methoxyphenyl)prop-2-enoic acid. (**17**) IG					
18.	6-Hydroxy-1a-(hydroxymethyl)-1a,1b,2,5a,6,6a-hexahydrooxireno[4,5] cyclopenta[1,2-c]pyran-2-yl 6-O-[(2E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoyl]-β-D-glucopyranoside. **(18)**IG	P.K.	Rhizomes	Hydro-alcoholic	------	[92]
19.	Scrophuloside. (**19**) IG					
20.	Veronicoside. (**20**) IG					

21.	Abeloside A. (**21**) IG					
22.	Abeloside B. (**22**) IG					
23.	Sylvestroside IV dimethyl acetal. (**23**) IG	P.K.	Stems	n- Butanol	------	[93]
24.	Sweroside. (**24**) IG					
25.	8-Epi-loganin. (**25**) IG					
26.	8-Epi-loganic acid. (**26**) IG					
27.	2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol. PG					
28.	2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromen-4-one. PG	P.S.	Rhizomes	95% Ethanol	--------	[30,97]
29.	Scroside G. PG					
30.	7 -Hydroxy-coumarine. (**27**)PG					[113]
31.	Cinnamic acid. (**28**) PG	P.S.	Rhizomes	n-Butanol	---------	
32.	Scroside H. **(29)**PPG	P.S.	Roots	Ethanol	----------	[114]
33.	Scroside I. **(30)**PPG					
34.	picrogentioside II. **(31)**SIG	P.S.	Roots	Ethanol	----------	[98]
35.	picrogentioside D. **(32)**SIG	P.S.	Roots	Methanol	----------	[115]
36.	Hebitol III. **(33)**HQG	P.S.		Methanol		
37.	Scrophuloside C. **(34)**HQG		Roots		----------	[116]
38.	Scrophuloside D. **(35)**HQG					
	Aromatic Ester					
39.	3- Methoxy-4-decanoxy benzoic acid. (**36**)					
40	3-Methoxy-4-tetradecanoxy-phenyl npent-7,9 -diene-11-al. (**37**)	P.K.	Rhizomes	Macerated with aqueous	------	[100]
41.	3- Methoxy-4-dodecanoxy- phenyl-n-pent-7,9-dien-11-al. (**38**)					
42.	3- Methoxy-4-dodecanoxy phenyl- *n*-pent-7,9-dien-11-al. (**39**)					
43.	3-Methoxy-4-tetradecanoxy-phenyl *n*-pent-7,9 -diene-11-al. (**40**)	P.K.	Rhizomes	Methanol	------	[91]
44.	3-Methoxy-4-decanoxy benzoic acid. (**41**)					
	Bis-iridoid					
45.	Saungmaygaoside A. (**42**)					
46.	Saungmaygaoside B. (**43**)	P.K.	Stems	n- Butanol	------	[93]
47.	Saungmaygaoside C. (**44**)					
48.	Saungmaygaoside D. (**45**)					
	Phenylpropenoids					
49.	Luteolin -7-O-β-D-glucoside.					
50.	Gallic acid.	P.S.	Rhizomes	95% Ethanol	---------	[30,97]
51.	Isoferulic acid.					
52.	Vanillic acid					
	Alcoholic compounds					
53.	Hexacosanol.	P.S.	Rhizomes	95% Ethanol	--------	[30,97]

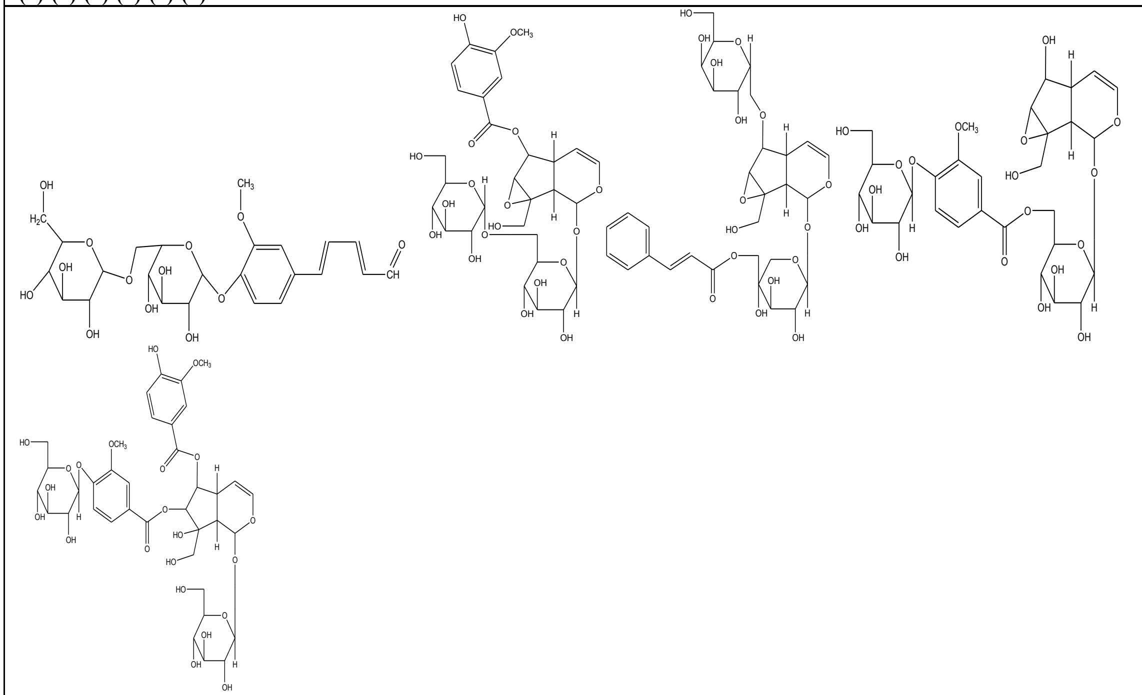
P.K.-*Picrorhizakurroa,* P.S.- *Picrorhizascrophulariiflora,* IG- Iridoid glycoside, AAG-Aromatic aldehyde glycoside, AADG- Aromatic aldehyde diglycoside, DPTG-Diphenolic tetra glycoside, PG- Phenolic glycoside, PPG-Phenylpropenoid glycoside, SIG- Secoiridoid glycoside, HQG-Hydroquinone glycoside

Picrorhiza plant. In *P. kurroa* there were 26 glycoside compounds (1-26), reported from the different parts of a plant like rhizomes, leaves and stems using different solvents like methanol, ethanol, n-butanol, chloroform, water, and methanol:water (1:1)[75,83-93] while in *P. scrophulariiflora* there are 12 glycosides (27-35), which have been reported from the different parts *viz*. roots, leaves and stems using different solvents like ethanol, butanol, (95 %) and ethyl acetate[29,30,83,91,94-99].

In aromatic esters, ester linkage is bonded directly to an aromatic system. Only from the roots of *P. kurroa* species 6 (36-41) aromatic ester have been isolated with



(1) (2) (3) (4) (5) (6)



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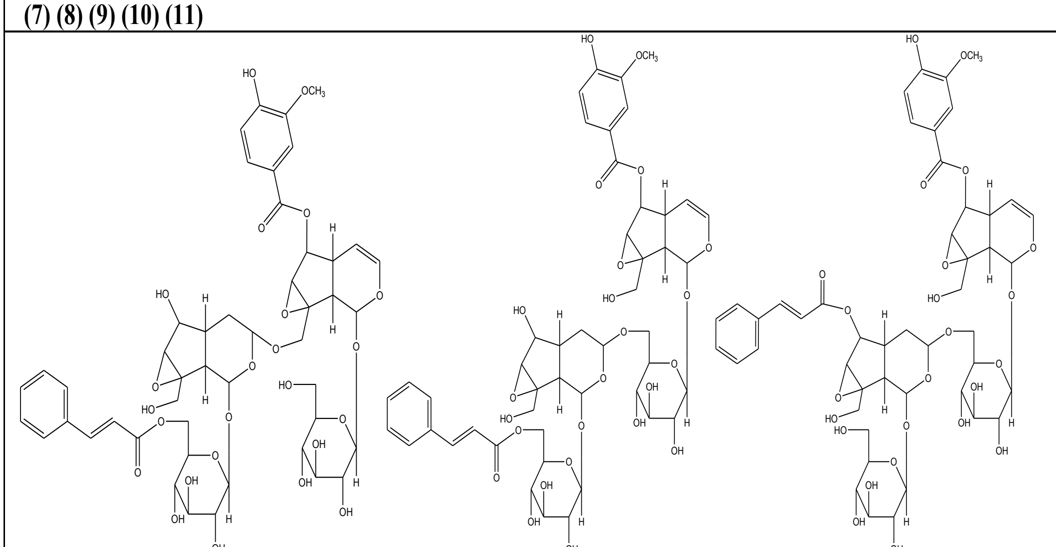

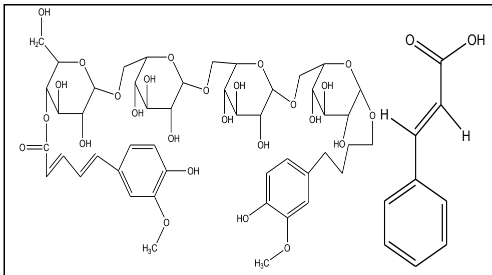

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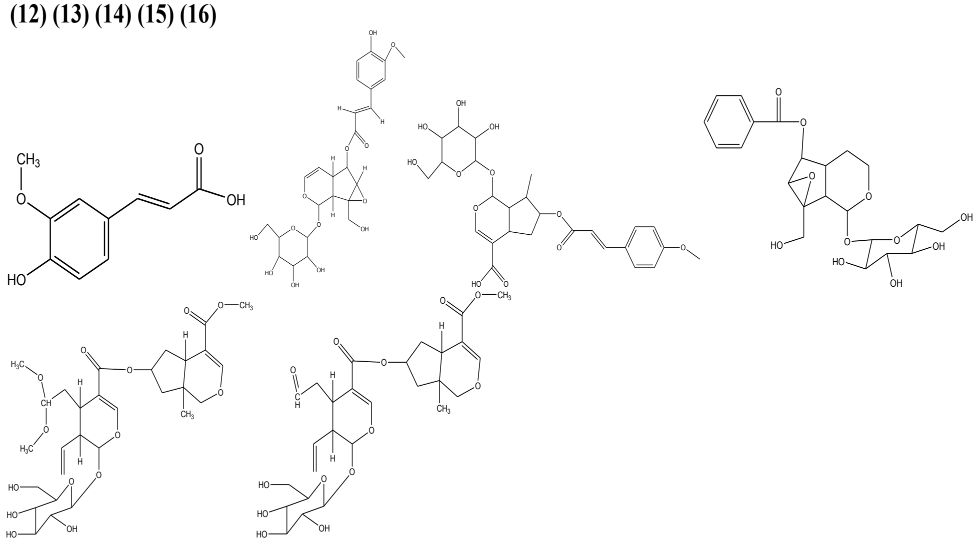
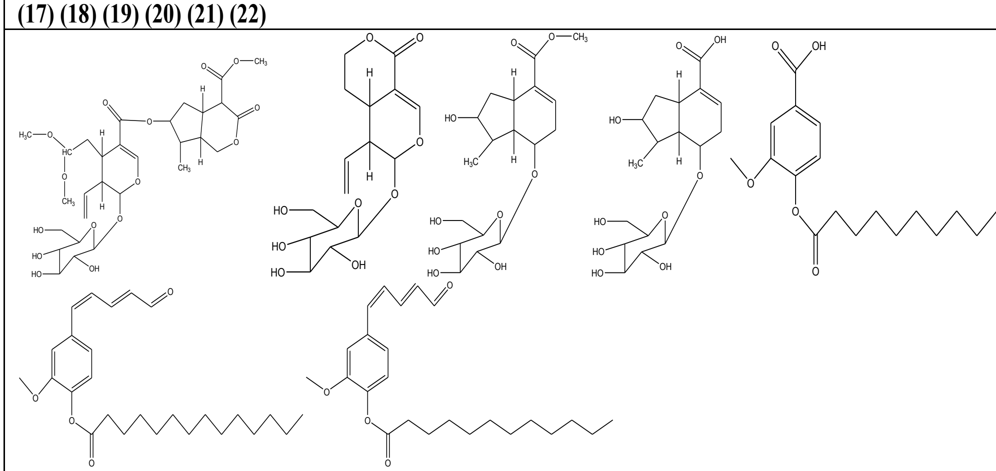
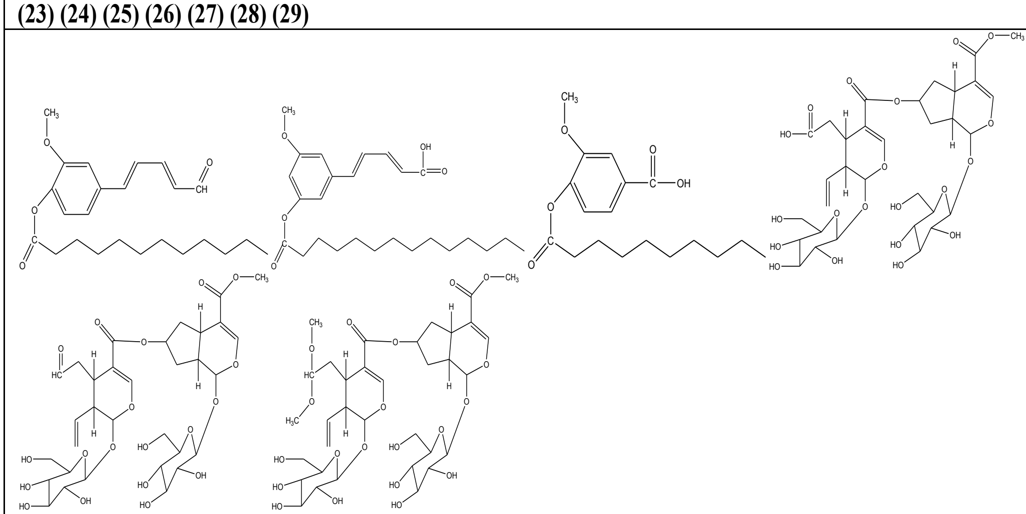


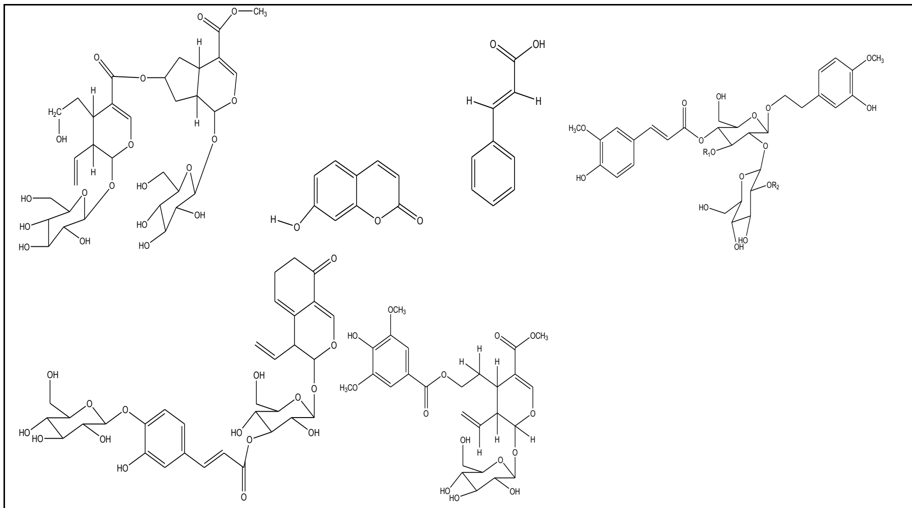
(17) (18) (19) (20) (21) (22)



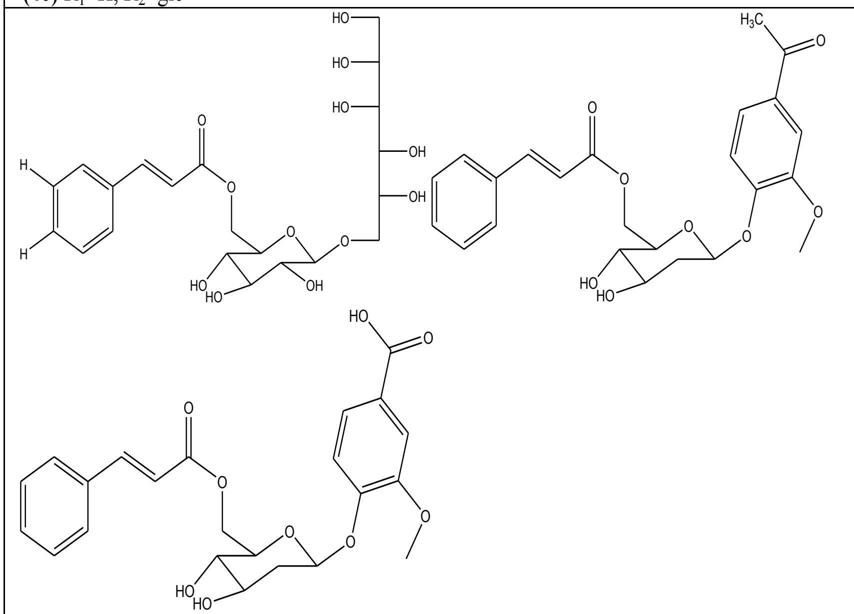
(23) (24) (25) (26) (27) (28) (29)



(30) (31) (32) (33) (34) (35)



(36) (37) (38) (39) R_1=rha, R_2=H **(41) (42)**
(40) R_1=H, R_2=glc



(43) (44) (45)

Fig. 1: Structures of isolated chemical compounds from genus *Picrorhiza*

water and methanol[91,100]. In case of bis-iriodids, 2 units are connected by a 1,3-dioxane group. Four bis-iriodids (42-45) have been isolated from the stems of *P. kurroa* with n-butanol[93]. From the roots of *P. scrophulariiflora* four phenylproponoid compounds have been isolated using (95 %) ethanol as a solvent[30,97].

Alcohols are organic compounds that contain at least one hydroxyl functional group bound to their aliphatic or aromatic substructure. One alcoholic compound has been isolated from the roots using ethanol as a solvent[30,97]. In the genus, *Picrorhiza* main phytochemical constituents are glycosides, which have been mainly isolated from rhizomes. The chemistry of glycosides from leaves, seeds, fruits and stems need to be explored.

Phytochemical analysis:

Many researchers used different analytical techniques like UV/Vis, IR, GC-MS, GC-FID, LC-MS, NMR, HPLC, HPTLC, UPLC, for the identification and characterization of new compounds from the genus *Picrorhiza*. A brief summary of *Picrorhiza* species using different analytical techniques are shown in Table 2.

Biological activities:

A wide range of biological activities of isolated chemical constituents and plant extracts, like antimicrobial[85], hepatoprotective[92], antioxidant activity[101], anticancer[86,102], antiarthritic[88], antidiabetic[87],

TABLE 2: PHYTOCHEMICAL ANALYSIS ON GENUS *PICRORHIZA*

Sr. no.	Plant species	Part of plant	Solvent used for extraction	Analytical Technique used	Result	References
1.	*P. kurroa*	Rhizomes	Methanol	HPTLC	Yield of extract is 40.63% (Rf 0.24)	[117]
2.	*P. kurroa*	Rhizomes	Methanol	HPTLC	Picroside-1 (Rf 0.51) and kutkoside (Rf 0.63)	[118]
3.	*P. kurroa*	Rhizomes	Ethanol:water	HPLC-UV	Picroside-1: 3.30%(w/w) Picroside-2: 4.90%(w/w)	[119]
4.	*P. kurroa*	Rhizomes	Diethyl ether	LC-ESI-MS/MS	cinnamic acid, ferulic acid, kuttoside, picroside-I, picroside-II, picroside-III, scrophuloside-A, and veronicoside.	[92]
5.	*P. kurroa*	Rhizomes	Ethanol:water	HPLC	Picroside-1: 9.57%(w/w) Picroside-2: 4.30%(w/w)	[90]
6.	*P. kurroa*	Rhizomesand leaves	Methanol:water	HPLC	Picroside-1: 98.3%(w/w) Picroside-2: 101.3%(w/w)	[89]
7.	*P. kurroa*	Roots	70% Ethanol	LC-ESI-MS/MS	Picroside III Apocynin , Pikuroside, Picroside IV, Vanillic acid , Picroside II, Kutkoside, Picroside I	[120]
8.	*P. kurroa*	Rhizomes	Ethanol	ILUAE	picroside I: 2.84% picroside II: 3.57% 6-O-E-feruloyl catalpol:2.20%	[121]
9.	*P. kurroa*	Rhizomes	Ethanol: water (mother extract) further fractionated using hexane,n-butanol, acetone, methanol and water	HPTLC	Mother extract: (25.6%w/w), hexane (9%w/w), DCM (31%w/w), n-butanol (23%w/w), acetone (11% w/w), methanol (16%w/w), and water (7%w/w). Cucurbitacin B Cucurbitacin D Cucurbitacin E Betulinic acid Picroside 2 Apocynin	[102]
10.	*P. scrophulariiflora*	Fruits	Ethanol	UPLC	Apocynin,Androsin, Picroside-1, Picroside-2, Picroside-3	[122]
11.	*P. kurroa and P. scrophulariiflora*	Roots	Methanol	HPTLC	Comparing Picroside-1 and Picroside-2 from two different species of picrorhiza Picroside-1(kurroa): 1.258% Picroside-1 (scrophulariiflora): 1.611% Picroside-2(kurroa): 0.481% Picroside-2 (scrophulariiflora): 0.613%	[123]

immunomodulating[103,104] have been reported either from the whole plant or specific homogenized parts to prove its medicinal importance (Table 3 and 4).

Iridoid glycosides (3-4) isolated from the rhizomes of *P. kurroa* have shown good antimicrobial activity (cup-plate method) against Gram-positive bacteria, *Bacillus subtilis*, *Staphylococcus aureus, Micrococcus luteus,* Gram-negative bacteria, *Pseudomonas aeruginosa*, *Escherichia coli* and fungal strains such as *Aspergillus niger, Candida albicans* and *Malasseiza furfur*[85,87].

The extract prepared using ethanol from the leaves of *P. kurroa* shown antimicrobial activity against the strains of *Staphylococcus*, *Pseudomonas,* and *E. coli* using the agar well diffusion method, and antifungal activity against the fungal strain viz *Candida albicans*, *Candida tropicalis*, *Trichophyton rubrum,* and *Penecillium marneffi*[105].

Iridoid glycoside (5) extracted from rhizome inhibited tumor invasion and migration of MCF-7 cells[86]. The rhizome extract of *Picrorhiza* has shown cytotoxicity in XTT assay in MDA-MB-435S (human breast carcinoma), Hep3B (human hepatocellular carcinoma), and PC-3 (human prostate cancer) cell lines[101]. Mallick *et al*.[102] studied the anticancer activity of the hydroalcoholic extract of *P. kurroa* and its fractions in breast cancer and cervix cancer cell lines. Oral administration of dichloromethane fraction significantly reduced tumor volume, tumor weight, and percent packed cell volume at a dose of 50 mg/kg, as compared with toxic control group.

Iridoid glycosides (5) extracted with water from the rhizomes of *P. kurro*a have shown antidiabitic activity. Compound (1) was orally administered to streptozotocin-induced diabetic rats at the doses of 100 and 200 mg/kg/day for 14 consecutive days. Plasma insulin levels were measured and the pancreas of the rat was subjected to histopathological investigations. There was evidence of regeneration of β-cells of pancreatic islets of picrorhiza extract-treated group in histopathological examinations. It increased the insulin-mediated translocation of glucose transporter from the cytosol to plasma membrane or increased glucose transporter type-4 appearance, which in turn facilitated glucose uptake by skeletal muscles in diabetic rats[87]. Aqueous ethanol extract of P. kurroa rhizomes demonstrated antibiabetic activity. Treatment with P. kurroa rhizomes extract (100 and 200 mg/kg) for 30 d significantly decreased high blood glucose and restored the normal levels of serum biochemistry[90].

Saponin and flavonoids have been isolated from ethanol extract of root of this plant, which exhibited antiasthmatic activity in bronchoconstriction and induced airway obstruction method. These saponins have been shown to possess mast cell stabilizing while flavonoids have been reported to possess smooth muscle relaxant and bronchodilator activity[106].

The ethanol extract of *P. kurroa* leaves were tested on a rat model of anemia induced by intraperitoneal injection of phenylhydrazine at 40 mg/kg for 2 d. The extract was given orally to the rats previously treated with phenylhydrazine at 100 and 200 mg/kg/d, resulted in the increased concentration of hemoglobin, red blood cell number, hematocrit, and reticulocytes rate[107].

Methanol extract of rhizomes of *P. kurroa* showed antioxidant activity in the superoxide anion radical scavenging method[108]. Antioxidant activity of the methanol extract was carried out using the radical scavenging assays (DPPH and OH), ferric reducing antioxidant property and thiobarbituric acid assay for testing inhibition of lipid peroxidation[101].

Kumar *et al.* 2016 studied the hydroalcoholic extract of rhizomes of *P. kurroa,* which showed antiarthritic activity. Additionally, *P. kurroa* rhizome extract significantly inhibited the expression of degrading enzymes, matrix metalloproteinases-3, and matrix metalloproteinases-9 in arthritic rats[88]. The methanol root extract of *P. kurroa* showed antitrypanosomal activity. It was carried out on a Vero cells and there was a complete kill of trypanosomes[109]. Picrorhizaosides D (4, IC_{50} 43.4 μM) and E (5, 35.8 μM) have shown strong hyaluronidase inhibitory activity[110].

Hydroalcoholic extract of rhizomes of *P. kurroa* demonstrated hepatoprotective activity at the dose levels of 50 and 100 mg/kg exemplified by significant restoration of liver enzyme levels and the antioxidant enzymes close control. *Picrorhiza kurroa* hydroalcoholic extract has ameliorative activity from tissue damage against induced hepatotoxicity[92].

Scrocaffeside A, a compound extracted from rhizomes of *P. scrophulariiflora* has shown immunomodulatory activity against spleen cell suspension of mice. Scrocaffeside A between 5 and 125 μg/ml stimulated proliferation of splenocytes and their response to polyclonal T cell mitogen concanavalin A and lipopolysaccharide[111].

Water extract of dried powdered rhizomes of *P. scrophulriiflora*, was given orally in doses of 20, 40, and 80 mg/kg/d for 4 w to rats fed with high-

TABLE 3: BIOLOGICAL ACTIVITIES OF ISOLATED CHEMICAL CONSTITUENTS

Solvent used	Chemical constituents	Strains/Test Animals	Doses and Control	Result	Reference
		Picrorhiza kurroa			
		Antimicrobial Activity			
Ethanol	[5-hydroxy-10-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy-3,9-dioxatricyclo[4.4.0.0^{2,4}]dec-7-en-2-yl]methyl 4-hydroxy-3-methoxybenzoate. (**3**)	*Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*	5 mg, 10 mg, 15 mg, 20 mg/75 µl and ciprofloxacin (5 mg/75 µl)	Effective against bacterial and fungal strain.	[85]
Methanol and water	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3-methoxy-4-[3-phenylprop-2-enoyl]oxybenzoatedihydrate. (**4**)	*Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginµosa, Bacillus subtilus*and *Escherichia coli; Candida albicans*and *Aspergillus nigar*	5 mg, 10 mg, 15 mg, 20 mg/75 µl and ciprofloxacin (5 mg/75 µl), Nystatin(5 µg/ml)	Methanolic extract is effective against all microbial while water extract is moderate against all bacterial and fungal strain.	[87]
		Anticancer Cancer			
Methanol and water	[6-[[(1a,1b,2,5a,6,6a)-6-hydroxy-1a-(hydroxymethyl)-2,5a,6,6a-tetrahydro-1bHoxireno[5,6]cyclopenta [1,3-c]pyran-2-yl]oxy]-3,4,5-trihydroxyoxan-2-yl] methyl-3 phenylprop-2-enoat. (**1**)	MCF-7 cell	5µg, 25µg, 50µg, 75µg, 100µg/ml and Paclitaxel (5µg/ml)	Inhibit tumor invasion and migration.	[86]
		Antidiabitic Activity			
Water	[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3-methoxy-4-[3-phenylprop-2-enoyl]oxybenzoatedihydrate. (**5**)	Rat	100 mg/kg, PkE, 200 mg/kg and glibenclamide (10mg/kg)	Increase the insulin mediated translocation and expression of glucose transporter-4.	[87]
		Hyaluronidase inhibitory activity			
Methanol	Picrorhizaoside-D (**11**) Picrorhizaoside-E (**12**)	Mast cell	0µg/ml; 3µg/ml; 10 µg/ml; 30 µg/ml 100 µg/ml and disodium cromoglycate, ketotifenumarate (0,3,10, 30,100µg/ml).	Shown similar or stronger activity than the antiallergic medicines.	[110]
		P. scrophulariiflora			
		Immunomodulatory activity			
Methanol	scrocaffeside A	Rat	0 µg/ml, 5 µg/ml, 25 µg/ml, 125 µg/ml and Con A(50µg/ml)	shown immunoenhancement effects on immune system	[111]

fat and high-sugar diet for 8 w to establish non-alcoholic steatohepatitis showed antifatty liver effect. Its mechanism appeared to be the regulation of lipid metabolism and reduction of insulin resistance, through inhibition of oxidative stress and inflammation[112-123].

Most of the biological activities have been studied extensively on *P. kurroa* as compared to *P. scrophulariiflora* as per the available literature, however, few biological activities, which have been reported were antimicrobial, high metabolic potential, immunomodulation, and therapeutic effect. There is an urgent need to explore the full potential of *P. scrophulariiflora*'s biological activities.

Future scope:

Traditionally rhizomes of *P. kurroa* have been used to cure a liver disorder, fever, lowering sugar level, cancer, asthma, jaundice, malnutrition, urinary

TABLE 4. BIOLOGICAL ACTIVITIES OF PLANT EXTRACTS

Solvent used	Strains/test animals	Doses and control	Result	Reference
		Picrorhiza kurroa		
		Antimicrobial Activity		
Ethanol; methanol	*Staphylococcus aureus, Pseudomonoas aeruginosa, Bacillus subtilus and E.coli; Candida albicans, Candida tropicalis, Trichophyton rubrum, Aspergillus nige*r and *Penecillium marneffi*	10, 25, 50, 100 mg/ml and ciprofloxacin (5 µg/ml), Nystatin (5 µg/ml)	Effective against all the selected bacterial and fungal strain.	[86,101]
Anticancer activity				
Methanol and water	MDA-MB-435S, Hep3B and PC-3 cells	15.625, 31.25, 62.5 and 125 µg/ml and XTT (0.6 mg/ml)	To induce apoptosis	[101]
Ethanol: Water (mother extract) further fractionated using n-hexane,dichloromethane, butanol, acetone, methanol and water	MCF-7, MDA-MB 231 and HeLa, SiHa cell lines	3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, and 500 μg/ml and MTT (5mg/ml)	Dichloromethane fraction of *P. kurroa*s hown effective anticancer activity	[102]
		Antidiabetic activity		
Water and ethanol	Rat	100 and 200 mg/kg and glibenclamide 10mg/kg	Helps in b-cell regeneration with enhanced insulin production and antihyperglycemic effects.	[90]
		Antiasthmatic Activity		
Ethanol	Guinea pig	25mg/kg And salbutamol (0.2mg/kg)	Reduce the inhibition of histamine and acetylcholine.	[106]
		Antianemic activity		
Ethanol	Rat	100 mg/kg/day and 200 mg/kg/day and phenylhydrazine (40mg/kg)	Increase the concentration of haemoglobin and increase the number of red blood cells	[107]
		Antioxidant activity		
Methanol and water	Rat	0.5 ml and EDTA	Methanolic extract is less efficient as compared to water in the scavenging of the radicals.	[101]
Methanol	Single cell of hepatocytes	0.7ml/kg and without P. kurroa extract (0.7ml/kg)	Elevated the malondialdehyde levels, decreased superoxide dismutase levels, glutathione levels and oxidative stress.	[108]
		Antiarthritis activity		
Water and Ethanol	Rat	2 mL/kg and Indomethacin (3 mg/kg)	Normalize redox status of synovium, suppression of pro-inflammatory cytokines, angiogenesis.	[119]
		Antitrypanosomal Activity		

Methanol	Rat	250 mg/kg and 1% gum acacia orally (2 mL/kg)	Reduction and killing of trypanosomes.	[109]
		Hepatoprotective activity		
Ethanol and Water	Rat	400 to 1500 mg/day and without extract	Significant restoration of liver enzyme level.	[92]
		P. scrophulariiflora		
		Therapeutic effect		
Water	Rat	20, 40 and 80 mg/kg/d and CMC (0.5%)	Significantly decreased total cholesterol and triglycerides assay in liver tissue and increased the Superoxide dismutase activity.	[112]

disorder, leukoderma, snake bite, piles, and rheumatic diseases. To verify the credibility of traditional uses, biological activities of rhizomes are yet to be tested in the areas of leukoderma, snake bite, and hemorrhoids. Similarly, the seeds and flowers of *P. kurroa* have been used traditionally to treat liver and upper respiratory tract disorders, fever, dyspepsia, chronic diarrhea, and blood purification but there is not much supporting data has been generated. Several traditional uses have been recorded to cure a liver disorder, choleretic, joint pain, asthma, jaundice, arthritis, and bacterial infection from the stems of *P. scrophulariiflora* but no scientific study has been carried out in this regard either.

In *P. kurroa* there are 29 chemical constituents (6-10,13-26,36-45), which have not been tested for any biological activities. Similarly, 9 chemical constituents (27-35) are isolated from *P. scrophulariiflora* but none were assessed for biological activities. Some biological activities of the genus *Picrorhiza* that differ from traditional uses are antianemic activity, antitrypanosomal activity, and high metabolic activity. Only some biological activities have buttressed traditional uses such as antimicrobial, hepatoprotective activity, antiasthmatic activity, arthritis activity, anticancer activity, antidiabetic activity.

Phytochemical studies on the genus *picrorhiza* have revealed at least 53 compounds in the last decade while the biological activities have been carried out on 8 compounds. These studies reveal antimicrobial, anticancer, antidiabetic, hyaluronidase inhibitory, immunomodulatory activities. On the basis of literature, it could be concluded that the rhizomes possessed a good profile of chemical constituents and biological activities. However more studies are required to explore about *P. scrophulariiflora*.

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Conflict of Interest:

The authors declare that they have read the policy and guidelines of the journal and there are no conflicts of interest.

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