CHAPTER IV

PICRORHIZA KURROA

4.1 Introduction

4.2 Review of Literature

4.3 Experimental

4.4 Results and discussion

4.5 Comparative studies of Picroside-I and Picroside-II of Picrorhiza Kurroa

4.6 References
4.1 INTRODUCTION

In recent years, many researchers have examined the effects of plants used traditionally by indigenous healers and herbalists to support liver function and treat diseases of the liver. In most cases, research has confirmed traditional experience and wisdom by discovering the mechanisms and modes of action of these plants as well as reaffirming the therapeutic effectiveness of certain plants or plant extracts in clinical studies. Several hundred plants have been examined for use in a wide variety of liver disorders. Just handfuls have been fairly well researched. The latter category of plants include: Pierorhiza kurroa (kutkin) (Fig 1), Silybum marianum (milk thistle), Curcuma longa (turmeric), Camellia sinensis (green tea), Chelidonium majus (greater celandine), Glycyrrhiza glabra (licorice), and Allium sativa (garlic). Pierorhiza kurroa have been shown to protect liver cells from a wide variety of insects including Amanita poisoning, carbon tetrachloride, ethanol, aflatoxin B1, thioacetamide and oxytetracycline.
Fig. 1- First publication on *Picrorhiza kurroa* by Royle in 1835.
4.2 REVIEW OF LITERATURE

Morphology

*Pierorhiza kurroa* is a well-known herb in the Ayurvedic system of medicine and has traditionally been used to treat disorders of the liver and upper respiratory tract, reduce fevers, to treat dyspepsia, chronic diarrhoea and scorpion sting. It is a small perennial herb from the Scrophulariaceae family, found in the Himalayan region growing at elevations of 3,000-5,000 metres. *Pierorhiza kurroa* has a long, creeping rootstock that is bitter in taste, grows in rock crevices and moist, sandy soil. The leaves of the plant are flat, oval, and sharply serrated. The flowers, which appear in June to August, are white or pale purple and borne on a tall spike; manual harvesting of the plant takes place in October to December. The active constituents are obtained from the root and rhizomes. The plant is self-regenerating but unregulated over-harvesting has caused it to be threatened to near extinction. Current research on *Pierorhiza kurroa* has focused on its hepatoprotective, anti-cholestatic, antioxidant and immune-modulating activity.

Distribution

*Pierorhiza kurroa* also known as “Kutki” is found in the North-Western Himalayan region from Kashmir to Kumaon and Garhwal regions in India and Nepal.
Vernacular names

Indian Name: Kutki and Kuru

Botanical Name: Pierorhiza kurroa

Other Names: Kutuka, Kuru and Kadu

Biological activities of Pierorhiza kurroa

Apocynin, present in the plant is a catechol that has been shown to inhibit neutrophil oxidative burst in addition to being a powerful anti-inflammatory agent, while the eucubitacins have been shown to be highly cytotoxic and possess antitumour effects. The hepatoprotective action of Pierorhiza kurroa is not fully understood but may be attributed to Pierorhiza's ability to inhibit the generation of oxygen anions and to scavenge free radicals. Pierorhiza's antioxidant effect has been shown to be similar to that of superoxide dismutase, metal-ion chelators, and xanthine oxidase inhibitors. In rats infected with malaria, Pierorhiza restored depleted glutathione levels, thereby enhancing detoxification and antioxidation, and helping to maintain a normal oxidation-reduction balance. In the same animal model, Pierorhiza also demonstrated an anti-lipid peroxidative effect. Like silymarin, Pierorhiza has been shown to stimulate liver regeneration in rats, possibly via stimulation of nucleic acid and protein synthesis. Pierorhiza's anti-inflammatory action is attributed to the apocynin constituent, which has been shown to have potent anti-inflammatory properties in addition to inhibiting oxidative burst in neutrophils. Although the mechanism is unclear, animal studies indicate that Pierorhiza's constituents exhibit a strong anticholestatic activity against a variety of liver-toxic substances,
appearing to be even more potent than silymarin. *Pierorhiza* also exhibits a dose-dependent choleretic activity, evidenced by an increase in bile salts and acids, and bile flow. *Pierorhiza* is not readily water-soluble and is therefore not usually taken as a tea. While it is ethanol soluble, the bitter taste makes tinctures unpalatable, so it is usually administered as a standardized (4% kuitkin) encapsulated powder extract. Typical adult dosage is 400 to 1500 mg/day, with dosages up to 3.5 g/day sometimes being recommended for fever. *Pierorhiza* root extracts are widely used in India with no adverse effects as have been reported.22-28

**Chemical constituents of *Pierorhiza kurroa***

Kuitkin is the active principal of *Pierorhiza kurroa* and is comprised of kuttoside and the iridoid glycoside picroside I, II and III. Other identified active constituents are apocynin, drosin, and eucurbitacin glycosides. Picroliv is a mixture of iridoid glycosides from the rhizomes of *Pierorhiza*.
R = H  Scroside A
R = Glc  Scroside B
Scroside C

1. \[ R_1 \quad R_2 \quad R_3 \quad \text{Cucurbitacin B} \]
2. \[ \text{OH} \quad \text{OH} \quad \text{OAc} \quad \text{Cucurbitacin Q} \]

Picein  Androsin  Veronicaside
4.3 EXPERIMENTAL

Materials and method

Melting points were determined by open capillaries in Mettler FP-800 melting point apparatus. The UV spectra were recorded on Hitachi 150-20 with data processor. The IR spectra were taken on Perkin-Elmer FT-IR 1760X. The $^1$H NMR (300MHz) and $^{13}$C NMR (300 MHz) were recorded on Bruker Avance-300 in specified deuterated solvents using TMS as internal standards. All chemical shift values were reported in $\delta$ (ppm). FAB-mass spectra on a Jeol 5x102 mass spectrometer with DA-6000 data system using argon (6kV, 10 mA) as the FAB gas and m-nitro benzyl alcohol as the matrix and EI-Mass spectra were recorded on Shimadzu GC-MS QP-2000A, in direct injection mode.

Medium Pressure Liquid Chromatography was applied on Buchi MPLC system (688 pumps, 684 fraction collector and 687 gradient former). Silica gel (230-400 mesh for flash chromatography, GLAXO) was used for MPLC column packing. Silica gel (60-120 Mesh Glaxo) was used for column chromatography. The fractions obtained after MPLC and column chromatographies were concentrated in Heidolph Laborota-4000 and Buchi-R-114 rotavapour. The extracts were dried in Heto-FD3 Dry winner lyphilizer.

TLC was performed on silica gel-G from Glaxo and s-d fine chemicals. Spots were visualized by spraying with suitable reagent after observation in iodine vapour. Aqueous sulphuric acid (10%) was generally used to find the presence of any compound. All Rf values were reported on TLC using silica gel-
G unless otherwise stated. Details of the solvent system and spraying reagents used during the chemical investigation are given below:

**Solvent System for Thin Layer Chromatography TLC (v/v)**

Picroside-I  
CH$_3$OH/DCM (04:96)

Picroside-II  
CH$_3$OH/DCM (06:94)

**Spraying and detection reagents**

1. Iodine
2. 10% Aqueous sulphuric acid
3. Libermann-Buchardt reagent

**Collection of plant material**

*Picrorhiza kurrooa* (whole plant) was collected from Palampur, Himachal Pradesh (Height: 1300 mts, 32°N, 76°E). The voucher specimen was identified by a taxonomist.

**Drying and grinding of plant material**

Freshly collected plant material was rinsed with water to remove soil and debris from the roots. The material was kept on blotting paper to absorb water and dried over night at room temperature. The foreign matters were removed manually. The material was dried in an oven at 35°C. The dried plant was powdered in a grinder. Mesh size of the sieve used was 2 mm.

**Extraction**

The powdered material of *Picrorhiza kurrooa* (whole plant) (4.0 kg) was taken in percolator with the help of a glass funnel. The material was soaked in
90% methanol (10 lts) for overnight. The extract was collected in a beaker and concentrated at 40.0°C under reduced pressure in rotavapour. The process was repeated four times. The extract was dried in a lyophilizer for 8.0 hr to remove last traces of solvent. The weight of the final extract was recorded (270.0 gm). The extract was stored in a refrigerator for further processing.

Isolation of pure compounds from n-butanol fraction by Medium Pressure Liquid Chromatography

Methanolic fraction (40.0 gm) was taken in an evaporating dish and dissolved in a small quantity of methanol. The extract was adsorbed on silica 100 gm (100-200 mesh, column chromatography) to form slurry. The MPLC column was packed with silica gel (100-200 mesh for flash chromatography) in hexane and the slurry was loaded at top of it. The elution was done with chloroform with increasing polarity using methanol.

Table 1: Polarity of eluting solvent

<table>
<thead>
<tr>
<th>Eluting solvent</th>
<th>Elution volume (ml)</th>
<th>Sub-fractions number</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Chloroform</td>
<td>100</td>
<td>1-30</td>
</tr>
<tr>
<td>2% Methanol in chloroform</td>
<td>100</td>
<td>31-60</td>
</tr>
<tr>
<td>4% Methanol in chloroform</td>
<td>100</td>
<td>61-90</td>
</tr>
<tr>
<td>6% Methanol in chloroform</td>
<td>100</td>
<td>91-120</td>
</tr>
<tr>
<td>8% Methanol in chloroform</td>
<td>100</td>
<td>121-150</td>
</tr>
<tr>
<td>10% Methanol in chloroform</td>
<td>100</td>
<td>151-180</td>
</tr>
</tbody>
</table>
The fractions were concentrated under vacuum at 40.0°C in rotavapour and monitored on TLC plates (polarity of TLC solvent was taken according to the fraction). The similar sub-fractions were mixed according to the TLC.

**Isolation of pure compounds from sub-fractions 31-120 by column chromatography**

The sub-fractions from 31-120 were mixed and concentrated under vacuum at 40.0°C in rotavapour. The extract was taken in an evaporating dish, mixed with 6 gm of silica gel (230-400 mesh) and made into slurry. Column was packed with silica gel (230-400 mesh) in hexane and the slurry was loaded on top of it. The elution was done with chloroform with stepwise increase in polarity with methanol.

**Table 2: Isolation of compounds with increasing polarity**

<table>
<thead>
<tr>
<th>Eluting solvent</th>
<th>Nos. of fraction</th>
<th>Volume (ml)</th>
<th>Wt. of the compound</th>
<th>Name of the compound</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% Chloroform</td>
<td>1-20</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.2% Methanol in chloroform</td>
<td>21-40</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>0.5% Methanol in chloroform</td>
<td>41-60</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.0% Methanol in chloroform</td>
<td>61-70</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.5% Methanol in chloroform</td>
<td>71-85</td>
<td>50</td>
<td>22.0 mg</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>2.0% Methanol in chloroform</td>
<td>86-100</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.5% Methanol in chloroform</td>
<td>101-120</td>
<td>50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3.0% Methanol in chloroform</td>
<td>121-140</td>
<td>50</td>
<td>19.0 mg</td>
<td>2</td>
<td>0.55</td>
</tr>
</tbody>
</table>
4.4 Results and Discussion

**Compound 1 (Picroside-I)**

IR Spectrum of Picroside-I indicated the presence of hydroxyl, aromatic ester and enol ether groups. It formed amorphous penta-acetate which was analyzed for C_{24}H_{35}O_{11} and its $^1$H NMR spectrum showed the presence of 5 acetyl, a cinnamoyl group, an olefinic proton as a doublet at $\delta$ 6.36 and an epoxy proton at $\delta$ 3.67. The $^1$H NMR data showed it to be an iridoid glucosidic ester of cinnamic acid.

The weight of the compound was 22.0 mg, $R_f$ was 0.45.

$\text{IR } \nu_{\text{max}} \text{ cm}^{-1}$: 3450 (OH), 1750 (Acetyl C=O) and 1650 (C=C).

$^1$H NMR (300 MHz, CD$_3$OD, $\delta$): 2.2-2.7 (2H, C-5 and C-9 H), 3.25-4.05 (8H, C-2', C-3', C-4', C-5', C-6, C-7 and C-10 2H), 4.45-5.22 (3H, C-1, C-4 and C-1'), 6.43(1H, dd, J=6 and 1.5 Hz, C-3H), 6.63, 7.85 (1H, d, J=16 Hz) and 7.46-7.95 (5H, C$_6$H$_5$CH=C=CH).

**Compound 2 (Picroside-II)**

IR Spectrum assignable to hydroxyl, aromatic ester and enol ether groups, was analysed for C_{23}H_{36}O_{13} and its $^1$H NMR spectrum showed the presence of 6 acetyl groups, one of which was phenolic, one methoxyl, an epoxy proton at $\delta$ 3.69, an olefinic proton at 6.40 as doublet and 3 protons of vanillloyl group between $\delta$ 7.2 and 7.74 and 8 protons between $\delta$ 3.25 and 4.05 assigned to protons on C-2', C-3', C-4', C-5', C-6, C-7 and C-6' with additional 3H of methoxy group in the case of kutkoside.
The weight of the compound was 19.0 mg, Rf was 0.55.

$\text{IR } \nu_{\text{max}} \text{ cm}^{-1}$: 3600-3200 (OH), 1700 (Acetyl C-O) and 1655 (C=C).

$^1$H NMR (300 MHz, CD$_3$OD, $\delta$): 2.34-2.9 (2H, C-5 and C-9 H), 3.25-4.05
(1H, C-2', C-3', C-4', C-5', C-6, C-7 and C-10 2H), 4.85-5.25 (3H, C-1, C-4,
and C-1'), 6.52 (1H, J=6 Hz, C-3) and 7.08, 7.47-8.1 (1H and 2H respectively
aromatic).

4.5 Comparative studies of Picroside-I and Picroside-II of *Picrorhiza Kurroa* from different locations

**Plant material**

Samples of *P. kurroa* were collected in the month of September-October, 2004 from different altitudes (ranging from 2600-4500 m) from western
Himalayan region. They were authenticated by Biodiversity Department, IHBT,
Palampur, H.P. Rhizomes of the plants were air dried at room temperature ($25^\circ$C
$\pm 2^\circ$C). Samples collected from different locations of western Himalaya region
are mentioned in the table below.
Table 3: Picroside-I and picroside-II contents of different locations of *P. karrooa* with altitude in triplicate by HPTLC method

<table>
<thead>
<tr>
<th>S No</th>
<th>Altitude (mts)</th>
<th>HPTLC % values in triplicate</th>
<th>Mean in %</th>
<th>Mean ±SE</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3200</td>
<td>P-I 1.668 1.675 1.669</td>
<td>1.672</td>
<td>1.672± 0.003</td>
<td>0.246</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 1.530 1.478 1.493</td>
<td>1.501</td>
<td>1.501± 0.016</td>
<td>5.720</td>
</tr>
<tr>
<td>2</td>
<td>3400</td>
<td>P-I 1.653 1.648 1.679</td>
<td>1.660</td>
<td>1.660± 0.013</td>
<td>1.003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 0.563 0.570 0.564</td>
<td>0.566</td>
<td>0.566± 0.003</td>
<td>0.673</td>
</tr>
<tr>
<td>3</td>
<td>3000</td>
<td>P-I 0.023 0.015 0.021</td>
<td>0.021</td>
<td>0.021± 0.002</td>
<td>3.839</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II - - -</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>2300</td>
<td>P-I 0.144 0.147 0.148</td>
<td>0.146</td>
<td>0.146± 0.0016</td>
<td>0.484</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 0.018 0.017 0.019</td>
<td>0.018</td>
<td>0.018± 0.0006</td>
<td>5.555</td>
</tr>
<tr>
<td>5</td>
<td>4000</td>
<td>P-I 0.546 0.539 0.531</td>
<td>0.538</td>
<td>0.538± 0.005</td>
<td>1.403</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 0.431 0.432 0.428</td>
<td>0.430</td>
<td>0.430± 0.0016</td>
<td>0.493</td>
</tr>
<tr>
<td>6</td>
<td>2500</td>
<td>P-I 0.176 0.186 0.179</td>
<td>0.180</td>
<td>0.180± 0.004</td>
<td>2.859</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 0.031 0.033 0.032</td>
<td>0.032</td>
<td>0.032± 0.0006</td>
<td>4.941</td>
</tr>
<tr>
<td>7</td>
<td>4145</td>
<td>P-I 0.684 0.692 0.697</td>
<td>0.691</td>
<td>0.691± 0.005</td>
<td>0.948</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 1.073 1.068 1.075</td>
<td>1.072</td>
<td>1.072± 0.003</td>
<td>0.336</td>
</tr>
<tr>
<td>8</td>
<td>1350</td>
<td>P-I 0.376 0.381 0.370</td>
<td>0.375</td>
<td>0.375± 0.004</td>
<td>1.484</td>
</tr>
<tr>
<td></td>
<td></td>
<td>P-II 0.379 0.368 0.375</td>
<td>0.374</td>
<td>0.374± 0.004</td>
<td>1.488</td>
</tr>
</tbody>
</table>
Preparation of crude extract

The air dried (25±2℃) rhizomes of P. kuruva (0.2 gm) were extracted in 10 ml of methanol. After 12 hr, they were filtered and dried. Dried extracts were re-dissolved in 1 ml of methanol and 5μl of each sample was spotted for quantification.

Preparation of standard solutions

Stock solutions of Pieroside-I and Pieroside-II (1.0 mg/ml) were prepared in methanol and different amounts (2-5 μl) of these were loaded on a TLC plate, using ATS 4 for preparing four point calibration curves.

High performance thin-layer chromatography

A Camag HPTLC system equipped with an automatic TLC sampler ATS 4, TLC scanner 3 and integrated software Win CATS version 1.2.3 was used for the analysis. HPTLC was applied on a pre-coated silica gel HPTLC 60F254 (20×20 cm) plate of 0.20 mm layer thickness. 5μl of each sample and the standards were applied on the plate as 6 mm wide bands with an automatic TLC sampler (ATS 4) under a flow of N₂ gas, 10 mm from the bottom, 10 mm from the side and the space between two spots was 6 mm of the plate (fig. 2).
Detection and Estimation of picroside I and picroside II

The linear ascending development was carried out in a CAMAG twin trough chamber (20 cm x 20 cm) which was pre-saturated with 25 ml mobile phase chloroform: methanol (82:18) for 30 min. at room temperature (25°C ± 2°C) and 50% ± 5 relative humidity. The length of chromatogram run was 8 cm. Subsequent to the development, TLC plates were dried in a current of air with the help of an air dryer in wooden chamber with adequate ventilation. The flow of air in the laboratory was maintained unidirectional (laminar flow, towards exhaust). Quantitative evaluation of the plate was performed in the absorption-reflection mode at 290 nm, using slit width 6×0.4 mm, data resolution 100.
µm/step and scanning speed 20 mm/s. The source of radiation utilized was
deuterium lamp emitting a continuous UV spectrum 190 and 400 nm.
Determination of the content of picroside in extracts was performed by the
external standard method, using pure P-I and P-II as standards. Each sample was
carried out in triplicate. A Camag Video Documentation system in conjunction
with the Reprostar 3 was used for imaging and archiving the thin layer
chromatograms. The object was captured by means of a high sensitive digital
camera with 4.0 M pixel CCD sensor and 3x optical zoom, model Power shot G2
( Canon, Singapore) (Fig. 2). A special digitizing board (frame grabber) assisted
in rapid processing via the personal computer system. Image acquisition
processing and archiving were controlled via win CATS software.
Fig. 3a - UV-Vis spectrum of standard P-I

Fig. 3b - UV-Vis spectrum of standard P-I and sample
Fig. 4a- UV-Vis spectrum of standard P-II

Fig. 4b- UV-Vis spectrum of standard P-II and sample
Calibration curve

Stock solutions of picroside-I and picroside-II (1.0 mg/ml) were prepared in methanol and 2-5 μl of these solutions were loaded on a TLC plate, using ATS4 for preparing four points calibration graphs.

Fig.5a- Linear Calibration curve of P-I,  Fig.5b- Linear Calibration curve of P-II
Validation of HPTLC densitometry method

Selectivity

Each compound was separated with baseline return as shown in fig.6a and fig.6b.

Fig-6a Standards P-I & P-II: Fig-6b P-I and P-II in the sample

HPTLC chromatogram of Picroside-I and Picroside-II

Accuracy and Recovery

To the pre-analyzed sample, 1.0 mg each of standard P-I and P-II were added and the mixture was analyzed by the proposed method. The experiment was conducted in triplicate to check, recovery and accuracy of the system. The results are summarized in Table 3, Table 4 showing the accuracy and recovery of the method as the mean values and the % CV values of P-I and P-II.
Table 4: Recovery study of Picroside I and II

<table>
<thead>
<tr>
<th></th>
<th>Amount of Picroside in rhizomes powder (mg)</th>
<th>Amount of picroside I added (mg)</th>
<th>Amount found in mixture (mg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picroside I</td>
<td>1.67</td>
<td>1.00</td>
<td>2.56±0.003</td>
<td>96.00</td>
</tr>
<tr>
<td>Picroside II</td>
<td>1.50</td>
<td>1.00</td>
<td>2.42±0.001</td>
<td>97.01</td>
</tr>
</tbody>
</table>

Limits of detection and quantification

The detection limit of individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantified as exact value. The quantification limit of individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), the blank methanol was spotted six times following the same method as explained. The signal to noise ratio was determined as signal to noise ratio 3:1 and 10:1 considered for LOD and LOQ, respectively. The limit of detection for P-I and P-II were 1.5 and 1.7 μg respectively and limit of quantification was 2 μg each (table 5).
Table 5: Linear regression equation and \( R_f \) for picrosides

<table>
<thead>
<tr>
<th>Picrosides</th>
<th>( R_f )</th>
<th>Regression equation</th>
<th>( r^2 )</th>
<th>SdV.</th>
<th>LOD (µg)</th>
<th>LOQ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Picroside I</td>
<td>0.60</td>
<td>( Y=3788.367+2638.326 )</td>
<td>0.995</td>
<td>3.00%</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Picroside II</td>
<td>0.43</td>
<td>( Y=16481.078+2939.720 )</td>
<td>0.994</td>
<td>1.81%</td>
<td>1.7</td>
<td>2</td>
</tr>
</tbody>
</table>

\( * \) Correlation coefficient

Precision validation

System precision was performed by spotting 6 samples each from the stock solution of P-I and P-II, (2000 µg) and were spotted on the silica gel 60F 254 plate and analyzed with the proposed method. The results are given in table 6.

Table 6: System precision studies of the developed method

<table>
<thead>
<tr>
<th>S. No</th>
<th>System precision</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area picroside-I</td>
<td>Area picroside-II</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2929.54</td>
<td>13317.98</td>
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</tr>
<tr>
<td>2</td>
<td>2984.36</td>
<td>13348.14</td>
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<td>3</td>
<td>2897.15</td>
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<td>4</td>
<td>2915.04</td>
<td>12486.47</td>
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<td>5</td>
<td>2934.46</td>
<td>13397.17</td>
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<td>6</td>
<td>2963.41</td>
<td>13387.65</td>
<td></td>
</tr>
<tr>
<td>STD DEV</td>
<td>60.78</td>
<td>63.79</td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td>2.069</td>
<td>0.476</td>
<td></td>
</tr>
</tbody>
</table>
Different compositions of the mobile phase were tested and desired resolutions of picroside-I and II with symmetrical and reproducible peaks were achieved by using mobile phase chloroform-methanol (82:18) (Figs. 6a and 6b). Peaks corresponding to picroside-I and picroside-II were at Rf 0.60 and 0.43 respectively. The methanolic extract, when subjected to HPTLC, showed the presence of P-I and P-II peaks. Comparison of the spectral characteristic of the peaks for standards of P-I and P-II and that of all the samples revealed the identity of P-I and P-II present in all the samples (Figs. 3a, 3b, 4a and 4b). The same sample of *P. kurroa* was used for the HPTLC analysis. The calibration curves (Figs. 5a and 5b) were linear in the range of 2 μg to 5 μg for P-I and P-II respectively. Linear regression equation R² and standard deviation are given in table 2. Peak purity tests of P-I and P-II were carried out by comparing UV-visible spectra of P-I and P-II in standard and sample track. For the examination of recovery rates, 1.0 mg each of stock solutions of pure P-I and P-II were added in one *Picrorbiza kurroa* extract and quantitative analysis was repeated three times. The average values of recovery were 96% and 97% for P-I and P-II respectively (table 4). In the method applied here, peaks corresponding to P-I and P-II were symmetrical and well separated from other spots.

After analyzing all samples collected from different altitudes in the month of October when the plant undergoes dormancy, it was found that % mean values of picroside-I and II were maximum at an altitude of 3200 m (P-I, 1.67% and P-II 1.50%). Further, it was observed that at an altitude of 3000 m no P-II was present. However, further studies are needed to ascertain the role of age.
and stage of harvest on picroside contents. Estimation of picroside content and bar diagram (Fig. 7) of all samples collected from different altitudes are given in table 1.

![Graph showing picroside content across different altitudes](image)

**Fig. 7.** Typical bar diagram of P-I and P-II in *P. kurroa* collected from different locations with different altitudes.

The HPTLC method developed here for the quantification of picroside-I and picroside-II in *P. kurroa* is simple, rapid, cost-effective and easily adaptable for screening and quantitative determination than any other analytical technique. Fifteen samples can be analyzed on a 20X20 cm. TLC plate was run in about 45 minute time after extraction of the samples. The present method is suitable for rapid screening of large number of plant samples for crop improvement under the plant-breeding programme.
4.6 REFERENCES


