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PHARMACOGNOSTICAL STUDY OF *PICRORHIZA KURROA* ROOT

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ABSTRACT: Introduction: *Picrorhiza kurroa* is a well-known plant used in Ayurvedic medicines belonging to the family, Scrophulariaceae. This herb has been traditionally used in treating liver disorders, upper respiratory tract disorders, reduce fever, scorpion stings, and treat dyspepsia and chronic diarrhea. **Experiment:** The macroscopy, microscopy, powder microscopy, physiochemical screening was done on *Picrorhiza kurroa* roots. TLC, HPTLC. **Results:** The macroscopy, microscopy, and powder microscopy study reveals the identification of *P. kurroa* root. The phytochemical studies showed the presence of secondary active constituents such as phenols, glycosides, *etc.*, TLC and HPTLC analysis showed that the chloroform fraction of *P. kurroa* has a higher number of peaks and spots compared to total extract.

INTRODUCTION: *Picrorhiza kurroa* (*P. kurroa*; Family Scrophulariaceae), a well-known herb in the Indian traditional Ayurveda system of medicine [1]. The leaves are 2-4 inches long, oval in shape with a sharp apex, flat, and serrate. The rhizome of picrorhiza is manually harvested in October through December. Like many species of medicinal plants, picrorhiza is threatened to near extinction due to over-harvesting. The *P. kurroa* has been used to treat disorders of the liver and is an important ingredient of many herbal preparations used for the treatment of liver ailments. Picroliv is a standardized iridoid glucoside mixture isolated from the roots and rhizomes of *P. kurroa*. It contains at least 60% of 1:1.5 mixture of picroside I and kutkoside and has been used as a hepato-protective agent in diseases such as jaundice [2].

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In addition, the nitric oxide scavenging activity, cardioprotective effect, anticancer effect, anti-diabetic activity, and anti-viral effect of *P. kurroa* extract have been reported. Oxidative stress is caused due to the imbalance between reactive oxygen species (ROS) generation and antioxidant defence of the body.

Increasing levels of ROS like hydroxyl radical (OH•), superoxide anion (O_2 •−) and hydrogen peroxide (H_2O_2) reduce the antioxidant levels such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) and glutathione (GSH) and further damage the cellular components like DNA, proteins, and lipids [3, 4]. Oxidative stress is well reported in ischemia, hypoxia, Parkinson's, Huntington's, and Alzheimer's diseases. Supplementation of a diet rich with antioxidant principles such as polyphenols and flavonoids can protect the cell from the damage of ROS. Herbal supplements rich in flavonoids, polyphenols, and terpenoids are used as a source of natural antioxidants to reduce or control symptoms associated with chronic or stress-

related illnesses in the liver, picroliv decreased the levels of lipid peroxides and hydroperoxides and facilitated the recovery of superoxide dismutase and glycogen [5].

Hepatotoxicity mainly caused by Galactosamine intoxication, which disrupts the membrane permeability of the plasma membrane, causing leakage of the enzymes to form the cell, which leads to the elevation of serum enzymes. Hence, a significant rise in the transaminase levels could be taken as an index of liver damage.

Galactosamine has great liver specificity compared to other toxic groups, such as paracetamol, acetaminophen, and carbon tetrachloride, because hepatocytes have high levels of galactokinase and galactose-1-uridylyltransferase and galactosamine does not affect other organs. Galactosamine induces hepatotoxicity with spotty hepatocytes, necrosis, and marked portal and parenchymal infiltration.

Galactosaimine also induces the depletion of uridine diphosphate (UDP) by increasing the production of UDP-sugar derivates, which causes inhibition of RNA and protein synthesis, leading to cell membrane deterioration [6, 7]. The focus of the current study was to evaluate the effects of chloroform fraction of *P. kurroa* root for its anti-oxidant and hepatoprotective activity using cell-line study

Methods of Collection and Extraction:

Collection: The root of *Picrorhiza kurroa* was purchased from the commercial shops in Paris, Chennai, Tamil Nadu. The identification was made on a botanical and morphological basis. The macro-scopical study of drugs was conducted with the naked eye. The size, shape, color, and organoleptic characters were observed, and the plant was confirmed on the basis of the literature description.

Extraction: The dried root was coarsely powdered. The powdered material was extracted by cold maceration method. The methanolic extract of roots was prepared by soaking the powdered material in the 90% methanol for 24 h. After that, the filtrate was collected and preserved. The marc is again is treated with methanol for 24 h. Again the filtrate was collected. Both the 1st filtrate and 2nd filtrate are mixed together.

The mixed filtrate is concentrated by evaporation, which produced brown color sticky residue, which was stored in the airtight container. The concentrated extract was weighed and further used for the experiments whenever required.

Fractionation: Methanol extract is fractionated with chloroform in the ratio of 1:4. That is, one part of extract is treated with 4 parts of chloroform in the separating funnel. The chloroform layer is collected. The chloroform fraction is concentrated by the evaporation method. The concentrated extract is used for the experiments.

Pharmacognostical Studies:

Macroscopic Evaluation or Organoleptic Study: Collected and authentified roots of *P. kurroa* were dried, and various organoleptic characters *viz.*, color, odor, taste, texture, fracture, and shape were studied [8, 9].

Microscopic Evaluation: Freehand sections of the root of *P. kurroa* were taken and stained using safranin. The section was observed under a compound microscope, and photographs were taken

Powder Microscopy: A pinch of *P. kurroa* root powder was taken in a watch glass and stain with 1-2 drops of staining agents such as iodine and wait for few minutes, then place a pinch on a glass slide and view under the microscope.

Phytochemical Screening: The phytochemical screening, such as the presence of alkaloids, tannin, glycosides, terpenoids, flavonoids, steroids, *etc*. are carried out as per the procedure given in the standard book [10].

Physiochemical Analysis: The physiochemical parameters such as Ash value, loss on drying, extractive value, swelling index, were carried out as per the procedure given in standard books.

TLC Study: The technique is used to separate the compounds and mainly used for the identification of the compounds [11].

Principle: Adsorption

Procedure:

For Total Methanolic Extract:

Mobile Phase:

1. Chloroform: hexane: acetic acid (50: 50:1).
2. Chloroform: ethyl acetate: acetic acid (50: 50:1).

Detecting Agent: Iodine vapors, vanillin – sulphuric acid

For Iridoid Glycosides:
Mobile Phase:

1. Chloroform: Methanol (8:2), (9:1).

Detecting Agent: Iodine vapors.

HPTLC Study: High-Performance Thin Layer Chromatography is used to establish reference "fingerprints" of herbs, which is one of the most powerful tools to link the botanical identity to the chemical constituent profile of the plant. HPTLC technique applied for the compilation of profiles pertaining to a varied range of constituents such as alkaloids, glycosides, terpenoids, flavonoids, saponins, resins, coumarins, plant hormones, antibiotics and number of other compounds of natural origin [12].

High-Performance Thin Layer Chromatography, also known under the synonym Planar Chromatography, is a powerful analytical technique with separation power, performance, and reproducibility superior to classical TLC. Today most HPTLC instruments are computer-controlled and can, therefore, offer dramatically improved reproducibility of the analytical result.

HPTLC Finger Print Chromatogram of Chloroform fraction:
Aim: To develop the HPTLC fingerprint of Chloroform fraction of *Picrorhiza kurroa* Chromatographic condition for HPTLC fingerprint.

Sample: Chloroform fraction of *Picrorhiza kurroa*.

Stationary Phase: HPTLC plates silica gel 60 f 254 nm and 365 nm

Mobile Phase: Chloroform: Methanol (8:2) Sample concentration: 10 mg/ml

Applied Volume: 2.0 µl, 4.0 µl and 6.0 µl

Development Chamber: Glass tank twin trough chamber

Development Mode: ascending

Scanning Wavelength: 254 nm and 365 nm

Documentation: 254 nm and 365 nm using CAMAG TLC scanner 3

RESULTS AND DISCUSSION:

Macroscopy:
Color: The rhizomes are deep grayish-brown in color, externally white, blackish internally with whitish wood.

Odor: Slight and unpleasant.

Taste: Bitter.

Size: 3 to 5 cm in length and 0.5 to 1 cm in diameter.

Shape: Cylindrical pieces with longitudinal wrinkles and annulations at the tip.

Features: Conical, buds, and stems along with the roots also constitute the drug. The roots are longitudinally wrinkled with transverse cracks. Fracture is tough. The result was shown in **Fig. 1**.

FIG. 1: *PICRORHIZA KURROA* ROOT

Microscopy: The transverse section of root showed 20-25 layers of cork consisting of tangentially elongated, submersed cells and cork cambium. The cortex is multilayered and vascular bundles are present in the cortex. The vascular bundles are surrounded by single layer endodermis of thick-walled cells. The secondary phloem is composed of phloem parenchyma and a few scattered fibers and 2-4 layered cambium. The secondary xylem consists of vessels, tracheids, xylem fibers, and xylem parenchyma. The tracheids are long, thick-walled, lignified, and more or less cylindrical. The xylem parenchyma is thin-walled, polygonal in shape, and center occupied by small pith consisting of thin-walled cells. It is a simple round to oval shape containing starch grains.

FIG. 2: MICROSCOPY OF *PICRORHIZA KURROA* ROOT

The periderm of the root constitutes 8-10 layers of thin-walled cork. The phelloderm cells are full of brownish contents.

The cortical cells are rounded with occasional inter-cellular spaces the secondary phloem is many-layered and xylem is made up of vessels, fibers and tracheids. Pith consists of thick parenchymatous cells. The result was shown in **Fig. 2**.

Powder Microscopy:

A **CORK CELL** **B** **CORTEX** **C** **MEDULLARY RAYS**

FIG. 3: POWDER MICROSCOPY OF *P. KURROA*

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE ROOT EXTRACT

Chemical tests	Chloroform fraction	Plant extract
Alkaloids		
Mayer's test	-	-
Dragendorff's test	-	-
Hager's test	-	-
Wagner's test	-	-
Carbohydrates		
Molish's test	-	-
Fehling's test	-	-
Benedict's test	-	-
Glycosides		
Anthrone test	+	+
Borntrager's test	+	+
Legal's test	+	+
Baljet's test	+	+
Keller-killiani test	+	+
Proteins		
Biuret's test	-	-
Million's test	-	-
Amino acids		
Ninhydrin test	-	-
Saponins		
Foam test	-	-
Flavanoids		
Shinoda test	+	+
Phenolic compounds		
Ferric chloride test	+	-
Lead acetate solution test	+	-
Tannins		
Ferric Chloride test	+	-
Lead Acetate test	+	-
Gelatin solution test	+	-

Microscopy of the powder **Fig. 3** showed Cortex cells were found circular to oval. Cork cells in transversely cut mode and in surface view were found to contain fragments of pitted vessels and tracheids and groups of cells with orange-colored content, medullary rays, xylem, cork cells are observed in the powder microscopy.

Preliminary Phytochemical Screening: The chloroform fraction of *P. kurroa* showed the presence of glycosides, flavonoids, phenols and tannins and the total plant extract of *P. kurroa* showed presence of glycosides and flavanoids. This indicates based on the polarity of the solvents the active constituents are solubilized; therefore higher the polarity of the solvent increases the solubility nature of active constituents. The result was showed in **Table 1**.

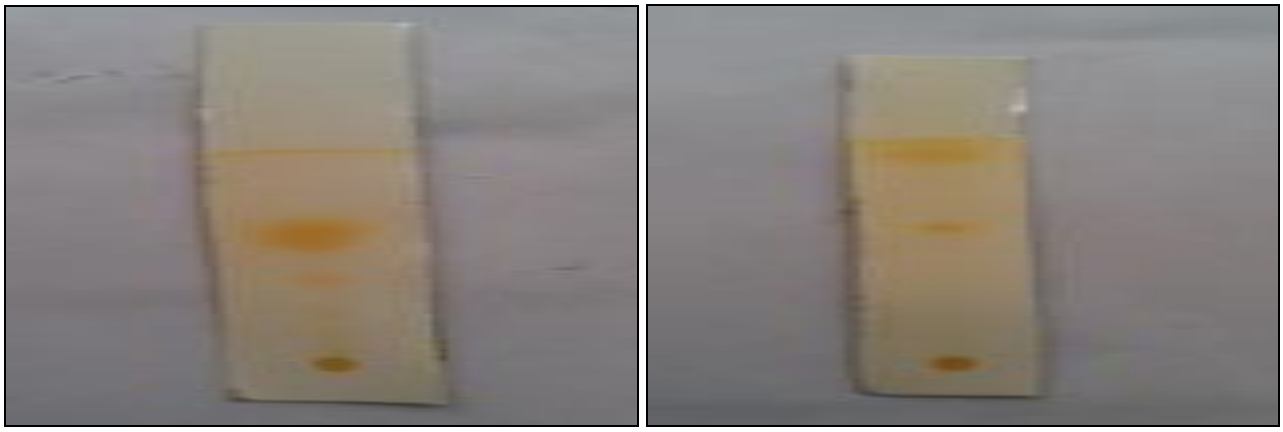
Physiochemical Analysis:

TABLE 2: PHYSIOCHEMICAL ANALYSIS STUDY

S. no.	Parameters	Results
1	Ash Values:	
	1. Total ash	6%w/w
	2. Acid insoluble ash	2%w/w
	3. Water soluble ash	1%w/w
2	Extractive Values:	
	1. Water soluble extractive	17.4%
	2. Alcohol soluble extractive	13.7%
3	Loss on drying	0.11
4	Swelling index	0.13

Report: The physicochemical analysis of *Picrorhiza kurroa* was carried out, and total ash was found to be 6% w/w, acid insoluble ash was found to be 2% w/w, water-soluble ash was found to be 1% w/w, alcohol soluble extractive was found to be 17.4%, water-soluble extractive was found to be 13.7%, loss on drying was found to be 0.11 and swelling index was found to be 0.13. The result was showed in **Table 2**.

TLC Study:



TLC OF TOTAL EXTRACT **TLC OF CHLOROFORM FRACTION**

FIG. 4: TLC OF *P. KURROA*

For Total Extract: R_f value of spot A was found to be 0.241, R_f value of spot B was found to be 0.354, R_f value of spot C was found to be 0.548.

For Chloroform Fraction: R_f value of spot A was found to be 0.627, R_f value of spot B was found to be 0.788.

The R_f value of chloroform fraction of *Picrorhiza kurroa* were found to be higher than the total extract of R_f values, At 254 nm, the samples showed 2-3 spots towards the baseline indicating the highly polar nature of the compounds. After dipping the plate in a vanillin-sulphuric acid solution, the plate showed different colored compounds indicating the presence of compounds with strong chromophoric groups.

This shows the solubility of the active constituents was based on the polarity of the solvent. The result was showed in **Fig. 4**.

HPTLC Fingerprint:

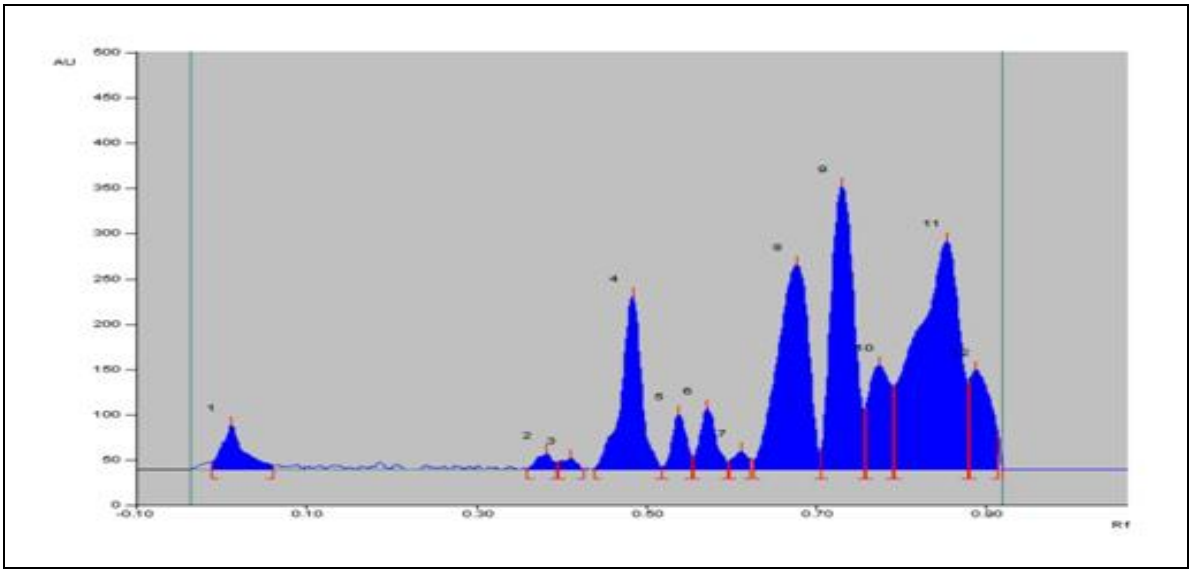


FIG. 5: FINGERPRINT OF CHLOROFORM FRACTION OF *PICORRHIZA KURROA*

TABLE 3: HPTLC OF CHLOROFORM FRACTION OF *PICORRHIZA KURROA*

Peak	Start R_f	Start height	Max R_f	Max height	Max %	End R_f	End height	Area	Area %	Assigned substance
1	-0.01	7.7	0.01	48.3	3.37	0.06	4.6	1104.8	2.88	Unknown *
2	0.36	0.8	0.38	18.3	1.28	0.39	8.5	290.3	0.76	Unknown *
3	0.39	8.7	0.41	12.3	0.86	0.42	0.0	198.5	0.52	Unknown *
4	0.44	1.5	0.48	190.7	13.33	0.52	3.0	3911.0	10.18	Unknown *
5	0.52	3.2	0.54	61.0	4.26	0.55	13.7	876.0	2.28	Unknown *
6	0.55	14.0	0.57	66.7	4.66	0.59	7.7	1109.2	2.89	Unknown *
7	0.59	8.5	0.61	20.2	1.41	0.62	12.0	307.3	0.80	Unknown *
8	0.62	10.9	0.68	226.0	15.79	0.70	17.9	7206.3	18.75	Unknown *
9	0.70	20.8	0.73	311.8	21.78	0.75	66.2	7272.7	18.93	Unknown *
10	0.76	68.1	0.77	114.7	8.02	0.79	91.8	2583.8	6.72	Unknown *
11	0.79	31.9	0.85	251.5	17.57	0.88	95.8	11187.3	29.11	Unknown *
12	0.88	97.0	0.89	109.6	7.66	0.91	39.8	2377.9	6.19	Unknown *

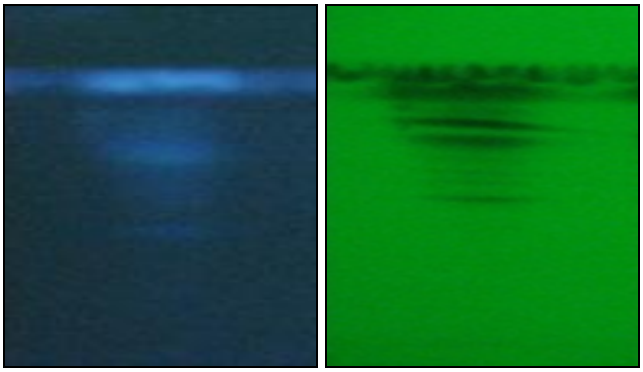


FIG. 6: HPTLC OF *PICRORHIZA KURROA* CHLOROFORM FRACTION

The HPTLC fingerprinting of chloroform fraction of *P. kurroa* showed the maximum number of UV active compounds, and that was detected in 254 nm and 365 nm.

Totally 12 peaks were observed with R_f value ranges from 0.06 to 0.91 at respective nm. Peak 10 shows the maximum height of 21.78%, with an area of 18.93%. Peak 3 shows a minimum height of 12.3% with an area of 0.52%, the result was showed in table 3 and **Fig. 5** and **6**.

CONCLUSION: Kutaki (*P. kurroa*) has been used in the indigenous system of medicine for a long time. Kutaki is considered to be a valuable bitter tonic and a favorite remedy in bilious dyspepsia accompanied by fever.

It is antipyretic, anthelmintic, and slightly laxative and is useful in asthma, blood troubles, burning sensation, piles, inflammations, ringworm. but some other species such as the root of *Picrorhhiza scrophularia* are sold in the drug market under the name kutaki or kuru so, there is a need to standardize the authentic source of genuine drug by using different parameters. This paper is an attempt of the author to generate identity and purity standards for *P. kurroa* to prevent its adulteration in the herbal drug market.

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