Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Phytochemical Characterization and Hepatoprotective Potential of *Plumbago Indica* Root Extract in Experimental Models

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ARTICLE DETAILS

Article history:
Received on 4 March 2026
Modified on 22 March 2026
Accepted on 25 March 2026

Keywords:
Plumbago Indica,
Qualitative,
Quantitative Phytochemical,
Antioxidant Activity,
Hepatoprotective.

ABSTRACT

The present study aims to analyze and compare the secondary metabolites of *Plumbago indica*, a medicinal plant, using both qualitative and quantitative approaches. Qualitative analysis will be performed through various chemical tests and chromatography techniques, including thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Quantitative estimation of total phenolic and flavonoid contents will be carried out using UV–Vis spectroscopy. Plant material will be collected, and extracts will be prepared using different solvents for subsequent analysis of secondary metabolites. Identified metabolites will be evaluated for their therapeutic potential and compared with the traditional uses of *Plumbago indica*. The hepatoprotective effect of the ethanolic extract will be assessed using *in vivo* models. The study is expected to provide valuable insights into the hepatoprotective potential of *Plumbago indica* ethanolic extract. The findings suggest that the extract can protect the liver from damage caused by free radicals generated during CCl_4 metabolism, highlighting its potential as a therapeutic agent for liver disorders.

INTRODUCTION

Medicinal plants have held a central place in ancient healing systems such as Chinese, Ayurvedic, and Egyptian medicine, and they remain widely used today for the treatment of numerous ailments[1]. Their therapeutic value is largely linked to phytochemicals—bioactive, non-nutritive compounds that, while not essential for human nutrition, provide protective and disease-preventive benefits. These compounds safeguard both the plants themselves and the humans who consume them. The wide range of biological activities exhibited by medicinal plants can be attributed to their diverse phytoconstituents, including vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, and alkaloids [1-4].

The liver is a vital organ with diverse and indispensable functions. It plays a central role in the metabolism of nutrients, including lipids, proteins, and carbohydrates, and is equally important in the excretion of waste metabolites [5, 6]. Moreover, as the first site of exposure to toxins absorbed from the intestinal tract, the liver is responsible for detoxification processes, including the metabolism and elimination of drugs and other xenobiotics [5, 6].

The liver also serves as a storage site for essential substances such as iron, vitamins, minerals, and glycogen. During fasting or periods of increased energy demand, hepatic glycogen is converted into glucose and released into the bloodstream to maintain blood glucose homeostasis [5, 7].

Liver disease remains a major global health challenge, particularly in developing countries. These disorders are broadly classified as non-inflammatory (hepatosis), inflammatory (acute or chronic hepatitis), and degenerative (cirrhosis or fibrosis). Common contributing factors include exposure to environmental toxins, heavy metals, alcohol abuse, malnutrition, viral infections, and indiscriminate use of medications. Such factors ultimately damage hepatocytes,

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leading to conditions such as hepatitis, jaundice, liver fibrosis, and alcoholic liver disease [7-10].

One clinical indicator of liver damage is an abnormal rise in blood cholesterol levels. Elevated concentrations of triacylglycerols (TAGs) and low-density lipoprotein cholesterol (LDL-C) significantly increase the risk of cardiovascular diseases (Dominiczak et al., 2005).

Table 1: Example of some drugs with hepatotoxicity effects

Drugs	Implication
Acetaminophen	It makes the cytochrome P-450-2E1 produce a toxic metabolite NAPQI that causes hepatic necrosis.
Amoxicillin	It moderates or brings about an increase in SGPT and SGOT levels, hepatic failure such as jaundice, acute cytolytic hepatitis, and hepatic cholestasis.
Chlorpromazine	It leads to infectious hepatitis with obstructive jaundice as a biomarker Isoniazid It elevates the serum transaminase level, severe and fatal hepatitis.
Ciprofloxacin	Elevation of SGOT alkaline phosphatase and SGPT levels occurs from cholestatic jaundice.
Diclofenac	It elevates AST and ALT levels, jaundice, fulminant hepatitis, and liver necrosis.
Erythromycin	It increases SGPT and SGOT concentration, and it also brings about hepatocellular hepatitis that are sometimes associated with it.
Fluconazole	It leads to hepatitis; it increases the transaminase level, fulminant hepatic failure, and cholestasis.
Oral contraceptives	Benign neoplasm, hepatic vein occlusion, and jaundice, but rarely neoplasm of the liver.
Rifampin	It leads to hepatitis, hyperbilirubinemia, and cholestasis.

MATERIALS AND METHODS

Plant Material

Fresh specimens of *Plumbago indica* roots were collected from the local region of Bhopal. The root sections selected for the study were first washed thoroughly under running tap water followed by rinsing with distilled water to remove surface impurities. The cleaned material was then air-dried at room temperature and subsequently shade-dried for 3–4 weeks to minimize the risk of contamination. After complete drying, the roots were powdered using an electric grinder, and the organoleptic properties such as color, odor, taste, and texture were assessed. The powdered material was then stored in an airtight container and preserved for subsequent phytochemical screening and biological evaluations [11].

Defatting of Plant Material

The shade-dried powdered roots of *Plumbago indica* were coarsely pulverized and subjected to maceration with petroleum ether for extraction. The process was continued until complete defatting of the plant material was achieved [11].

Extraction by Soxhlation Method

The defatted powdered roots of *Plumbago indica* were subjected to successive Soxhlet extraction using solvents of increasing polarity, namely chloroform, ethyl acetate, ethanol, and water. Following extraction, each solvent was concentrated by evaporation at temperatures exceeding their respective boiling points. The resulting dried extracts were weighed, and the percentage yields were calculated [12].

Phytochemical Screening

Preliminary phytochemical screening of the extracts was carried out using standard qualitative protocols to identify the presence of bioactive constituents. The tests were based on characteristic color changes or precipitate formation observed upon the addition of specific reagents to the extract solutions [11].

Total Phenol Determination

The total phenolic content (TPC) of the extracts was determined using the method developed by Parkhe and Bharti. In this procedure, 2 mL of extract or standard solution was mixed with 1 mL of Folin–Ciocalteu reagent (diluted 1:10 v/v with distilled water) and 1 mL of sodium carbonate solution (7.5 g/L). The mixture was vortexed for 15 seconds and allowed to stand for 10 minutes for color development. Absorbance was recorded at 765 nm using a UV–visible spectrophotometer. The TPC was quantified using a calibration curve prepared with gallic acid, and results were expressed as milligrams of gallic acid equivalent (GAE) per 100 mg of extract [13].

Total Flavonoids Determination
The total flavonoid content (TFC) of the extracts was estimated using the method developed by Parkhe and Bharti. In this procedure, 1 mL of a 2% aluminum chloride ($AlCl_3$) solution was added to 3 mL of the extract or standard solution. The reaction mixture was allowed to stand at room temperature for 15 minutes. Absorbance was then measured at 420 nm using a UV–visible spectrophotometer. The TFC was determined from a calibration curve prepared with quercetin, and the results were expressed as milligrams of quercetin equivalents (QE) per 100 mg of extract [13] .

Total Alkaloids Determination
The total alkaloid content (TAC) of the extracts was estimated using a spectrophotometric method. Briefly, 1 mg of plant extract was dissolved in methanol, followed by the addition of 1 mL of 2 N HCl, and the solution was filtered. To the filtrate, 5 mL of bromocresol green solution and 5 mL of phosphate buffer were added in a separating funnel. The mixture was successively extracted with 1, 2, 3, and 4 mL of chloroform, and the combined chloroform fractions were collected in a 10 mL volumetric flask and diluted to volume with chloroform. A standard calibration curve was prepared using atropine solutions at concentrations of 40, 60, 80, 100, and 120µg/mL processed in the same manner as the extracts. Absorbance of both test and standard solutions was measured at 470 nm against a reagent blank using a UV–visible spectrophotometer. The TAC was calculated from the calibration curve and expressed as milligrams of atropine equivalents (AE) per 100 mg of extract [14].

Experimental Animals
Wistar rats (200–250 g) were housed in groups of six (n = 6) under controlled conditions of temperature (25 ± 2°C) and humidity (55–65%), with a 12-hour light/dark cycle. The animals had free access to standard rodent feed and water. Prior to experimentation, rats were acclimated to the laboratory environment for 7 days. All experiments were conducted between 08:00 and 15:00 hours in a noise-free room. Separate groups of six rats were used for each set of experiments. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), established under the Ministry of Environment and Forests, Government of India, New Delhi, India, for the regulation and oversight of experimental animal use [15].

Experimental Design and Treatment Protocol
Rats were acclimated to laboratory conditions for seven days, maintained under a 12-hour light/dark cycle, a temperature of 25 °C, and 55% humidity. During this period, the animals were provided with a standard basal diet and had ad libitum access to water (Khan et al., 2012) [16].

CCl_4-Induced Hepatotoxicity
Group –I: Normal control (0.5% CMC 1 mL/kg, p.o.)

Group –II: Rats were administrated with CCl_4 (2 mL/kg b.wt.)

Group –III: Rats were administered carbon tetrachloride (CCl_4) at a dose of 2 mL/kg body weight and silymarin at 10 mg/kg. Silymarin, widely recognized for its hepatoprotective effects, exhibits antifibrotic, anti-inflammatory, and antioxidant properties. It exerts its protective action by stabilizing cellular membranes and enhancing protein synthesis, making it a standard reference compound in liver-protective studies globally.

Group –IV: CCl_4 (2 mL/kg b.wt.) and an ethanolic extract of the root of *Plumbago indica* L (100 mg/kg) were administered to rats.

Group –V: CCl_4 (2 mL/kg body weight) and 200 mg/kg of an ethanolic extract of *Plumbago indica* L root were administered to rats. [16]

Biochemical Analysis
Blood samples were collected from the ocular venous plexus via the retro-orbital bleeding method to assess various biochemical parameters. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase (ALP) were determined using an automated analyzer and commercially available kits (Roche). Additionally, the serum concentrations of malondialdehyde (MDA) and total protein were measured following the methods described by Ullah [17].

RESULTS AND DISCUSSION
Extracts of *Plumbago indica* were prepared using four different solvents: chloroform, ethyl acetate, ethanol, and distilled water. The extractive yields were calculated and expressed as percentage (%) values. In case of ethyl acetate extract the percentage yield observed to be 0.925 %. For chloroform & aqueous extract it is 1.162% and

3.280% respectively. The greatest percentage yield of 5.050 % is with ethanol extract [12, 18, 19]. The identified that methanolic extract gave the highest yield (16.83%) while the yields were lower for the acetone (14.31%), ethanol (9.25%), petroleum ether (4.14%), and water (6.35%) extracts, respectively [16]. From this result it is clear that Ethanol or Methanol can be considered as best solvent in which higher percentage yield can be obtained.

Table 2: % Yield of *Plumbago indica* extracts

S. No.	Extracts	% Yield (W/W)
1.	Chloroform	1.162
2.	Ethyl acetate	0.925
3.	Ethanol	5.050
4.	Aqueous	3.280

Result of Phytochemical Screening

Alkaloids, tannins, saponins, flavonoids, phenols, steroids, carotenoids, and other phytochemicals found in medicinal plants have a variety of disease-prevention properties [11, 20-21]. These chemical compounds from plants have major preventative effects, particularly in the areas of wound healing, anti-inflammatory, anti-diabetic, anti-aging, antibacterial, antiparasitic, antidepressant, and anti-parasitic properties [20-23]. They play a significant role in plants' ability to withstand stress as well as the accumulation of numerous significant bioactive substances in fruits and vegetables.

In order to detect the existence of particular phytoconstituent a number of qualitative phytochemical tests are performed. The chloroform extract gave positive results for flavonoids and proteins only. While, ethyl acetate extract contains alkaloid, flavonoid, proteins and carbohydrate. The aqueous extract gave positive result for occurrence of alkaloid, Flavonoid, proteins and saponin. The maximum numbers of phytoconstituents are present in ethanolic extract. Apart from diterpenes all the phytoconstituents are present [11, 18, 24].

Table 3: Result of Phytochemical screening

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**				
	Hager's Test:	-ve	+ve	+ve	+ve
2.	**Glycosides**				
	Legal's Test:	-ve	-ve	+ve	-ve
3.	**Flavonoids**				
	Lead acetate Test:	+ve	+ve	+ve	+ve
4.	**Diterpenes**				
	Copper acetate Test:	-ve	-ve	-ve	-ve
5.	**Phenol**				
	Ferric Chloride Test:	-ve	-ve	+ve	-ve
6.	**Proteins**				
	Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	**Carbohydrate**				
	Fehling's Test:	-ve	+ve	+ve	-ve
8.	**Saponins**				
	Froth Test:	-ve	-ve	+ve	+ve

Table 4: Results of estimation of total phenolic, flavonoids and alkaloid content

S. No	Extracts	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Chloroform	-	0.75	-
2	Ethyl acetate	-	0.66	3.40
3	Ethanol	5.58	0.95	3.96
4	Aqueous	-	0.60	3.55

Separation and Identification of Phytoconstituents by Thin Layer Chromatography (TLC) of *Plumbago Indica*

Table 5: Identification of Gallic acid by TLC

***Plumbago Indica* (Gallic acid)**			
S. No.	**Mobile Phase** Toluene: Ethyl acetate: Formic acid (7:5:1)	**Spot Distance**	**Rf Value**
1.	**(Gallic acid)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=1 No. of spot at normal light =1	Long – 3.1 Short – 3.1 Normal light – 3.1	Long – 0.62 Short – 0.62 Normal light – 0.62
2.	**(Ethanolic extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 5 No. of spot at short UV=5 No. of spot at normal light =3	Long – 1.9, 2.7, 3.5, 4.3, 4.5 Short – 2.5, 2.8, 3.5, 4.3, 4.5 Normal light – 2.8, 4.3, 4.5	Long – 0.36, 0.52, 0.7, 0.84, 0.9 Short – 0.49, 0.57, 0.69, 0.85, 0.9 Normal light – 0.56, 0.85, 0.9

Table 6: Identification of Quercetin by TLC

***Plumbago Indica* (Quercetin)**			
S. No.	**Mobile Phase** Toluene: Ethyl acetate: Formic acid (5:4:1)	**Spot Distance**	**Rf Value**
1.	**(Quercetin)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=1 No. of spot at normal light =1	Long – 3.0 Short – 3.0 Normal light – 3.0	Long – 0.6 Short – 0.6 Normal light – 0.6
2.	**(Chloroform extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 5 No. of spot at short UV=6 No. of spot at normal light =3	Long – 2.9, 3.9, 3.6, 4.9, 4.7 Short – 2.5, 2.8, 3.3, 3.7, 4.3, 4.7 Normal light – 3.3, 3.7, 4.8	Long – 0.58, 0.64, 0.72, 0.86, 0.64 Short – 0.5, 0.56, 0.66, 0.74, 0.86, 0.94 Normal light – 0.66, 0.74, 0.96
3.	**(Ethyl acetate extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 7 No. of spot at short UV=5 No. of spot at normal light =5	Long – 2.9, 3.2, 3.4, 3.6, 4.2, 4.6, 4.9 Short – 2.8, 3.3, 3.8, 4.6, 4.9 Normal light – 1.8, 2.2, 3.3, 4.4, 4.7	Long – 0.58, 0.64, 0.68, 0.72, 0.84, 0.92, 0.98 Short – 0.56, 0.66, 0.76, 0.92, 0.98 Normal light – 0.36, 0.44, 0.66, 0.88, 0.94
4.	**(Ethanolic extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 4 No. of spot at short UV=5 No. of spot at normal light =2	Long – 3.2, 4.3,4.7, 3.6 Short – 2.7, 2.9, 3.2, 3.7, 4.7 Normal light – 3.2, 4.7	Long –0.64, 0.86, 0.94, 0.72 Short – 0.54, 0.58, 0.64, 0.74, 0.94 Normal light –0.64, 0.94
5.	**(Aqueous extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 0 No. of spot at short UV=1 No. of spot at normal light =0	Long – 0 Short – 3.2 Normal light – 0	Long – Short – 0.64 Normal light –0

Results of Estimation of Total Phenolic, Flavonoids and Alkaloid Content

From the overall results obtained by performing various test one can noticed that the ethanolic extract contain good amount of Phenol, Flavonoid & alkaloid. The chloroform extract only have flavonoid content with value 0.74mg/100mg of dried extract. The total phenolic content is only estimated in ethanolic extract *of plumbago indica* which is found to be

5.55mg/100mg of dried extract. The flavonoid and alkaloid content in ethanolic extract is 0.93mg/100mg & 3.94mg/100mg respectively. Ethyl acetate contains flavonoid and alkaloid in amount of 0.68mg/100 mg & 3.38 mg/100 mg respectively. In case of aqueous extract total flavonoid and alkaloid present is found to be 0.59 mg/100mg and 3.50mg/100mg respectively [25-27].

Table 7: Identification of Colchicine by TLC

***Plumbago Indica* (Colchicine)**			
S. No.	**Mobile pHase** Toluene: Dichloromethane: Methanol (3:3:3)	**Spot Distance**	**Rf Value**
1.	**(Colchicine)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=0 No. of spot at normal light =0	Long – 2.2 Short – 0 Normal light – 0	Long – 0.44 Short – 0 Normal light – 0
2.	**(Ethyl acetate extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 5 No. of spot at short UV=4 No. of spot at normal light =2	Long – 2.2, 3.2, 3.6, 4.5, 5.0 Short – 2.2, 3.4, 4.4, 5.0 Normal light – 2.0,4.5	Long –0.44, 0.64, 0.72, 0.9, 1.0 Short – 0.44, 0.68, 0.8, 8.1 Normal light –0.4, 0.9
3.	**(Ethanolic extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 3 No. of spot at short UV=2 No. of spot at normal light =0	Long – 2.1, 3.3, 4.5 Short – 2.1, 4.5 Normal light – 0	Long – 0.42, 0.66, 0.9 Short – 0.42, 0.9 Normal light –0
4.	**(Aqueous extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=0 No. of spot at normal light =0	Long – 2.4 Short – 0 Normal light – 0	Long – 0.48 Short – 0 Normal light –0
5.	**(Aqueous extract)** Dis. Travelled by mobile phase= 5cm No. of spot at long UV= 1 No. of spot at short UV=0 No. of spot at normal light =0	Long – 2.4 Short – 0 Normal light – 0	Long – 0.48 Short – 0 Normal light –0

Identification of Marker Compound (Quercetin) by HPLC

Each of the standard drug solutions were injected 3 times and the mean peak area of drug was calculated and plotted against the concentration of the drug. The regression equation was found out by using this curve.

Table 8: Preparation of calibration curve of Quercetin

S. No.	Concentration (µg/mL)	Mean AUC
1.	5	440.521±24
2.	10	862.62±15
3.	15	1332.75±19
4.	20	1747.26±32
5.	25	2157.516±26

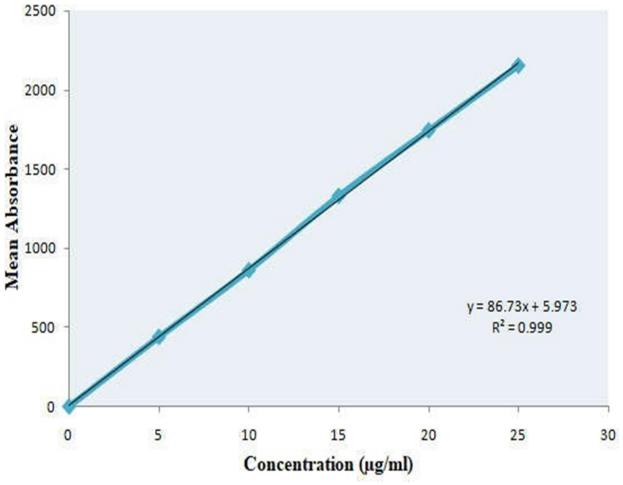

Figure 1: Calibration Curve of Quercetin

Table 9: Characteristics of analytical method derived from the standard calibration curve

Compound	Linearity Range µg/mL	Correlation Co-efficient	Slope	Intercept
Quercetin	5-25	0.999	86.73	5.973

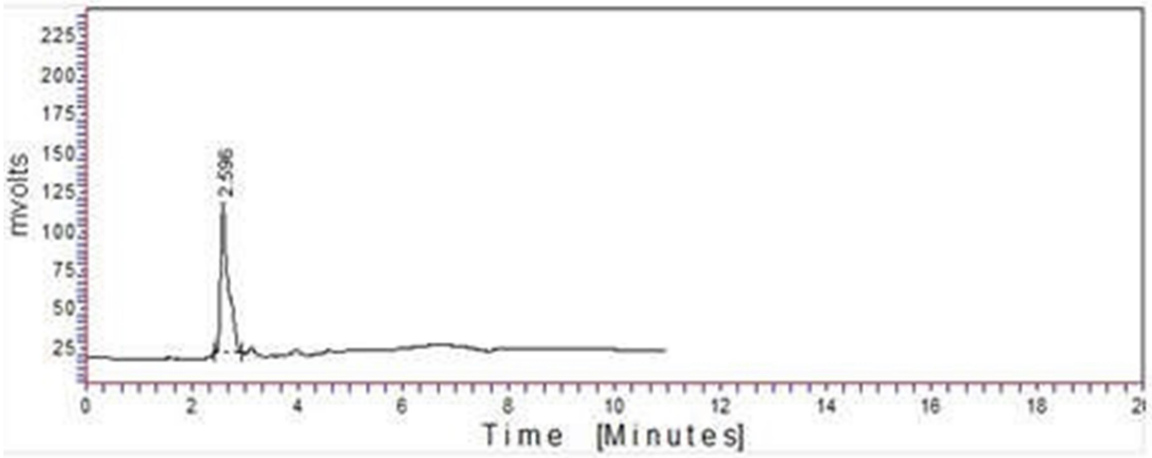

Figure 2: Chromatogram of Standard Quercetin

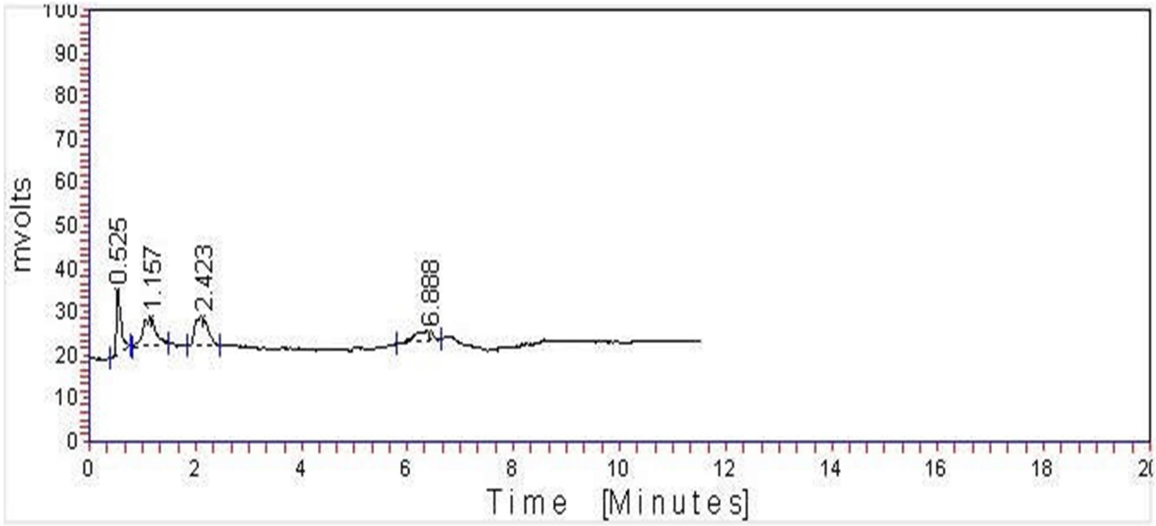

Figure 3: Chromatogram of Ethanolic Extract of *Plumbago indica*

Table 10: Quantitative Estimation of Quercetin in Extract

S. No.	Extract	RT	Area	% Assay
1.	Ethanolic extract	2.423	101.54	0.110

Results of *In-Vivo* Hepatoprotective Activity

Acute Toxicity Studies:

Acute oral toxicity studies and dose selection were carried out in accordance with the OECD draft guidelines 423, as approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy Wistar rats of either sex, weighing 200–250 g, were used to determine the median lethal dose (LD_{50}) of the phytosomal formulation [15, 28].

Rats were randomly selected, individually marked for identification, and housed in their cages for 7 days to acclimate to laboratory conditions. During the acute toxicity study, the phytosomal formulation administered at doses up to 200 mg/kg body weight did not produce any toxic symptoms. The animals displayed normal behavior with no observable neurological or behavioral abnormalities, and no mortality was recorded throughout the 14-day observation period [15, 28, 29].

Body weights were recorded at the beginning and end of the study. There were no significant changes in body weight among all treatment groups.

Biochemical Analysis

At the end of the experimental period, serum transaminases (SGPT, SGOT), cholesterol, and triglyceride levels were significantly elevated in the CCl_4-treated control group. Treatment with the ethanolic extract of *Plumbago indica* roots (100 and 200 mg/kg, p.o.) resulted in a significant reduction in SGPT, SGOT, cholesterol, and triglyceride levels compared to the control. In the silymarin-treated group (10 mg/kg, p.o.), SGPT levels were also significantly reduced relative to the CCl_4 control group [30, 31].

Table 11: Mean Body Weight Change

Groups	Drug	Dose	Body weight (g)	
			Onset of study	End of study
I	Normal Control	0.5% CMC 1 mL/kg, p.o.	218.6 ± 3.8	235.1 ± 3.6
II	Control (CCl4)	2 mL/kg b.wt.	227.3 ± 3.6	229.4 ± 3.9
III	CCl4+Silymarin	10 mg/kg p.o.	236.1 ± 3.9	246.1 ± 4.2
IV	CCl4+ ethanolic extract of *Plumbago indica* L	100 mg/kg p.o.	235.7 ± 4.1	245.3 ± 3.8
V	CCl4+ ethanolic extract of *Plumbago indica* L	200 mg/kg p.o.	242.5 ± 4.3	249.7 ± 4.4

CONCLUSION

Extracts from *Plumbago indica* were prepared and evaluated using a sequential solvent extraction method with chloroform, ethyl acetate, ethanol, and water. Among these, the ethanolic extract exhibited the highest percentage yield, followed by ethyl acetate, chloroform, and water. Phytochemical screening revealed that the ethanol extract contained the greatest diversity of bioactive constituents.

Quantitative analyses of total flavonoid, phenolic, and alkaloid contents were performed using standard methods. The results demonstrated a concentration-dependent increase in absorbance with increasing extract concentration. The highest total flavonoid and phenolic contents were observed in the ethanol extract, whereas the alkaloid content was greatest in the ethyl acetate extract, with ethanol and aqueous extracts showing comparatively lower levels.

Thin layer chromatography (TLC) was employed to confirm the presence of flavonoids, phenols, and alkaloids, using quercetin, gallic acid, and colchicine as reference standards, respectively. The Rf value of colchicine matched that of the ethyl acetate extract, confirming its presence. Additionally, high-performance liquid chromatography (HPLC) was used to identify quercetin as a marker compound in the extracts.

It is noteworthy that drug abuse is a significant contributing factor to liver damage, highlighting the relevance of hepatoprotective studies using *Plumbago indica*.

Drug-induced hepatotoxicity can manifest in a spectrum of conditions, ranging from asymptomatic elevations in liver enzymes to severe liver failure. Carbon tetrachloride (CCl_4) is commonly used in experimental studies as a hepatotoxin to evaluate the hepatoprotective potential of plants and natural products. The mechanism of CCl_4-induced liver injury in animal models is well established. In this study, administration of CCl_4 to rats caused significant liver damage, evidenced by elevated serum levels of AST, ALT, and ALP. Hepatocellular injury disrupts normal cellular function, increases membrane permeability, and leads to the leakage of enzymes into the extracellular space.

Pre- and post-treatment with the ethanolic extract of *Plumbago indica* roots significantly attenuated the severity of CCl_4-induced hepatic damage. The hepatoprotective effect is likely associated with the high flavonoid content of the ethanolic extract, which may help restore antioxidant enzyme levels and prevent extensive liver and pancreatic injury. The protective activity of the extract appears to involve the neutralization of reactive oxygen species (ROS), thereby preventing degeneration of the liver parenchyma. These findings indicate that the ethanolic extract of *Plumbago indica* roots possesses potent antioxidant properties and can safeguard the liver against free radical-mediated damage resulting from CCl_4 metabolism.

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