RESEARCH ARTICLE

Isolation, Characterization and Antioxidant Activity of *Plumbago indica* L. Extract

Gayatri Khosla[1], Vikram Sharma[2], Vikesh K. Shukla[1*]

[1]*Department of Pharmaceutics, Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India*
[2]*Galgotias College of Pharmacy, Greater Noida, Uttar Pradesh, India*

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ABSTRACT

The studies aimed to evaluate the preliminary parameter and Antioxidant potential of *Plumbago indica*. Standardization using various analytical techniques was also performed. *P. indica* was collected and studied for preliminary analysis and antioxidant activity and analyzed using the standard protocol using different analytical techniques. DPPH free radical scavenging activity was used to assess the antioxidant potential. The microscopy indicated the presence of periderm, cork and cortex, sclereids, cork, secondary xylem and medullary rays bordered pitted vessel, calcium oxalate crystal, starch grain, and cork with resin cell. The total ash, moisture, water-soluble, acid soluble ash, water soluble extractive and the alcohol soluble extractive value was 1.388, 5.146, 0.674, 0.19, 3.88, 3.6%, respectively. The presence of variety of phytoconstituents was discovered during phytochemical analysis. The total phenol content (TPC) and total flavonoid content (TFC) of the ethanolic and hydroalcoholic extract was 533.83 and 46.667 mg/gm, respectively and 132.66 and 219.00 mg/gm, respectively. The thin-layer chromatography (TLC), fluorescence in suspension hybridisation (FLASH), ultraviolet (UV), fourier transform infrared spectroscopy (FT-IR) and high-performance thin-layer chromatography (HPTLC), analysis illustrated the presence of Plumbagin and antioxidant activities because of its bioactive compounds. The ethanolic extract gives higher antioxidant potential by DDPH free radical scavenging activity. The findings of this study may be useful in establishing botanical and analytical grades for the root of *P. indica.*

Keywords: Antioxidants, Cold maceration extraction, Microscopy, Pharmacognostic, Phytoconstituents, *Plumbago indica L.*

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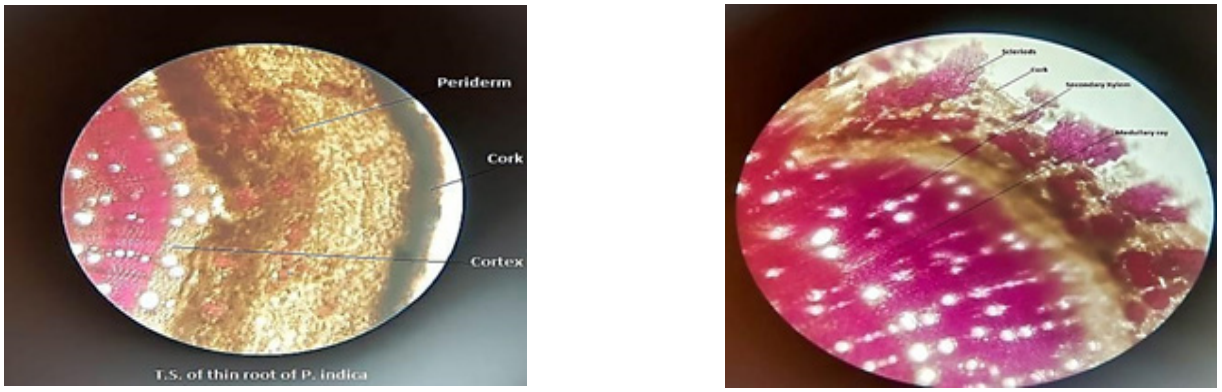
INTRODUCTION

Plumbago is a genus in the *Plumbaginaceae* family, which includes 24 genera and 400 species. *Plumbagoamplexicaulis Oliv., Plumbago aphylla Bojer ex Boiss., Plumbago auriculata Lam., Plumbago ciliate Engl., Plumbago caerulea Kunth, Plumbago dawei Rolfe, Plumbago europaea L., Plumbago glandulicaulis Wilmot-Dear, Plumbago indica L., Plumbago madagascariensis M. Peltier, Plumbago montis-elgonis Bullock, Plumbago pearsonii (L.) Bolus, Plumbago pulchella Boiss., Plumbago stenophylla Wilmot-Dear, Plumbagoscandens L., Plumbago tristis Aiton, Plumbago wissii Friedr.* and *Plumbago zeylanica L.* are the *Plumbago* genus. This genus is distinguished by its hairy calyx.[1,2] *P. indica* is a half-woody, erect or spreading plant that has been used in ayurveda, siddha, unani, and homeopathy for centuries.[2] This species originated in India's Sikkim and Khasi hills and spread to neighbouring countries such as Sri Lanka.[3] *P. indica* is grown as a medicinal and ornamental plant throughout the temperate and tropics zones.

P. indica is a perennial-herb or small shrub that thrives in warm tropical climates. These small shrubs can reach a height of 1.0–1.5 m and have erect, trailing, or climbing stems. From the base, the stem is either simple or branched.[3] The leaves are about 10 cm long, ovate-elliptic in shape, simple, alternately arranged with an entire margins, and exstipulate. The leaf's base tapers into a short, somewhat clasping petiole.[2]

Plumbagin (5-Hydroxy-2-methyl-1, 4-naphtho-quinone) is a natural bioactive organic compound isolated from *P. indica* roots.[4] Plumbagin is currently extracted from *P. indica* roots. It is a simple hydroxynaphthoquinone with commercial significance due to its vast spectrum of pharmacological activities.[5] Plumbagin is a yellow needle-shaped crystal slightly soluble in hot water and in alcohol, chloroform, benzene, acetone, and acetic acid. Plumbagin's melting point ranges between 78–79°C.[3]

**Author for Correspondence: vkshukla@amity.edu*



(a) (b)

Figure 1: A. Transverse section of thin root shows the presence of periderm, cork and cortex B. Transverse section of thick root shows the presence of sclereids, cork, secondary xylem and medullary rays

MATERIALS AND METHODS

Chemicals and Reagents

Methanol and Petroleum ether were procured from Avantor Performance Materials (RANKEM) Pvt. Ltd, Gurgaon, India. Follinciocalteu's reagent, Ascorbic acid and Rutin were procured from Merck, Pvt. Ltd. Gallic acid were obtained from Himedia, Pvt. Ltd. Sodium carbonate were obtained from Sd fine chemicals, Pvt. Ltd. Ethanol used were of Analytical grade. All of the other reagents, solvents, and chemicals used were laboratory grade.

Plant Material

The *P. indica's* roots was collected from Khari baoli, Delhi. The roots of the *P. indica* were washed, shade dried and powdered. The specimen was authenticated by submitting it to the Raw Material Herbarium and Museum Delhi (RHMD). Dr. Sunita Garg of CSIR NISCAIR New Delhi authenticated the entire plant in the CSIR NISCAIR New Delhi lab and voucher no. is NISCAIN/RHMD/consult/2020/3696-97.

Microscopic Evaluation

Microscopy evaluations were done on a qualitative basis. All investigations were studied on a compound microscope.

Root Microscopy

Microtome was used to make transverse sections of the root and powder for qualitative microscopic investigation. The staining method was followed exactly as directed. Staining was used to investigate several distinguishing traits.

For qualitative microscopic evaluation, In this study, the root was dipped in water for 24 hours for softening and microscopic slides were arranged as indicated by standard procedures.[6,7] Staining reagents phloroglucinol and concentrated HCl (1:1) were applied according to the standard method. Sample was taken in a clean slide of glass and with the help of drawing brush, section was transferred. One to two drops of glycerine water was added on the section with a dropper and with the help of forecep section was mounted and observed under a compound microscope at projection 45X and 100X (Figure 1).[8]

Powder Microscopy

Shade dried roots were passed through sieve no. 60 after being coarsely pulverized to analyze the various types of tissues or structures, which were then viewed through a microscope. Powder microscopy was performed according to conventional procedures (Figure 2).[9,10]

Extraction

Cold Maceration

The plant's crude material was extracted using cold maceration; plant samples were collected, washed, and dried properly. Dried powder of plant sample (420.200 gms) were extracted with organic solvents having different polarities *viz.* Pet. Ether Chloroform Ethanol Acetone Methanol Aqueous and hydroalcohalic and allow to stand for 4–5 days each. The extract of the plant was filtered to remove all unextractable matter Excess moisture was removed from the extract before it was collected in an airtight container. Extraction yield of all extracts were calculated (Table 1).[9]

Pharmacognostical Evaluation

Pharmacognostical evaluation includes the ash value percentage and extractive values were evaluated according to the official methods[11] and subsequently validated. Calibration models based on partial least squares were accurate for the prediction of total ash (R2=0.914; standard errors of prediction=0.373[12] and as per WHO guidelines on quality-control methods for herbal plant materials (Table 2).

Ash Value Determination

To evaluate the drug's ash concentration, about 2 grams of powder was placed in a pre-ignited and weighed silica crucible. After that, the crucible was gradually burned to remove all carbon. The total ash content of the crucible was determined by weighing it after cooling, and the ash was then examined to determine the acid insoluble and water soluble ash. The percentage of total ash was calculated using the air dried sample as a standard,[11] and subsequently validated. Calibration models based on partial least squares were accurate

Table 1: Results of colors and percentage yield (in gms and %) from the various extracts acquired from root of *P. indica*

S. no.	*Solvent*	*Colour of extract*	*Theoretical weight (in gms)*	*Yield (in gms)*	*Percentage Yield (%)*
1.	Pet. Ether	Yellow	420. 200	0. 472	0. 112
2.	Chloroform	Red	416. 53	0. 63	0. 151
3.	Ethanol	Brown	412. 24	9. 04	2. 192
4.	Acetone	Red	413. 33	7. 385	1. 786
5.	Methanol	Red	411. 9	18. 775	4. 558
6.	Aqueous	brown	396. 42	3. 74	0. 943
7.	Hydroalcoholic	Brown	404. 67	5. 56	1. 374

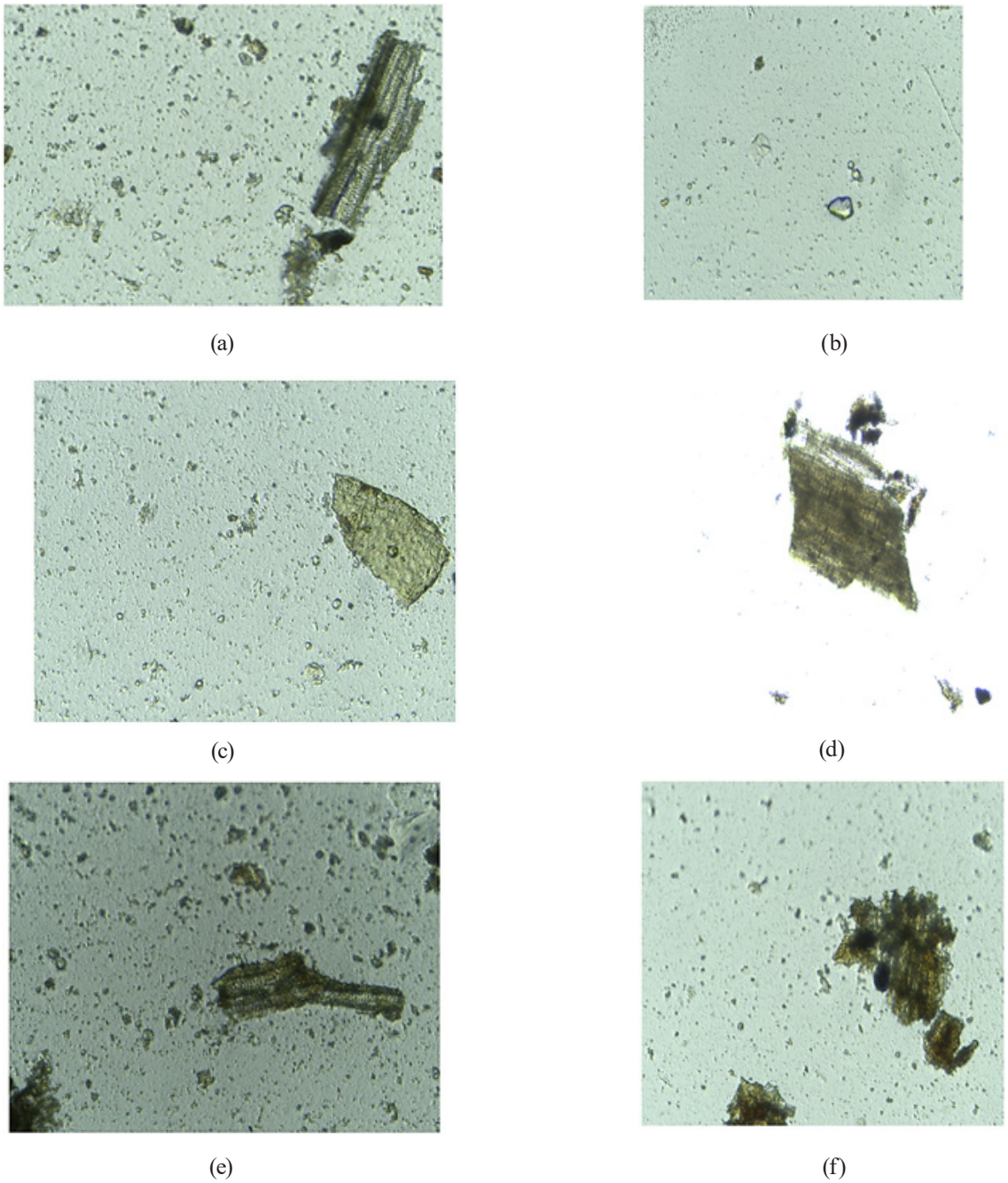


Figure 2: Powder Microscopy- A) bordred pitted vessel B) calcium oxalate crystal and starch grain C) epidermis with starch D) medullary rays radially cut E) pitted fiber F) scattered parenchyma, stone cell

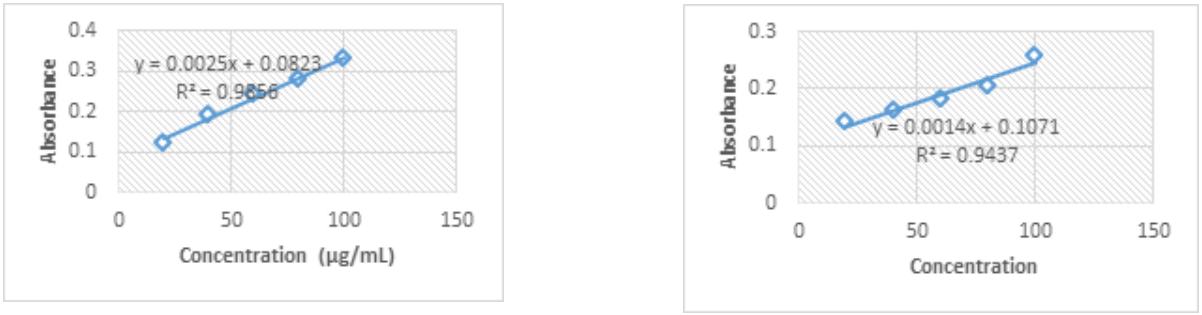
Table 2: Results of Pharmacognostical evaluation

S. no.	*Name of the assay*	*Observation (%)*
1	Total ash content	1. 388
2	Loss on Drying	5. 146
3	Water soluble ash	0. 674
4	Acid soluble ash	0. 19
5	Water extractive value	3. 88
6	Alcoholic extractive value	3. 6

for the prediction of total ash (R2=0. 914; standard errors of prediction=0. 373.

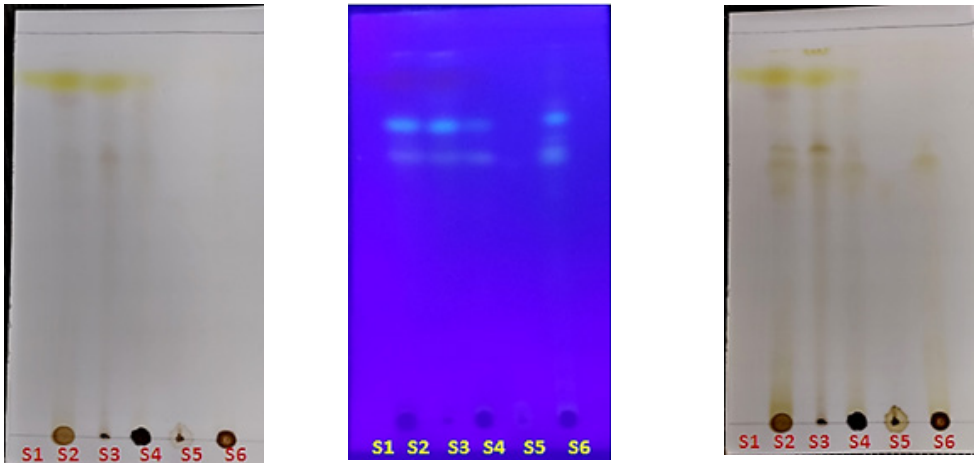
Moisture Content Determination

The LOD method (loss of weight on drying)was used to evaluate the moisture content. For this, 1–2 grams of drug (powdered root material) was collected and evenly distributed till a steady weight was achieved. The sample was dried for a predetermined amount of time at a temperature range of ± 2°C. As a comparison to air dried material, the amount of moisture found in the sample of plant was estimated.[12]



(a) (b)

Figure 3: Standard curve for different concentration of Gallic acid (a) and Rutin (b)



(a) (b) (c)

Figure 4: TLC profile of plumbaginindica extracts: Solvent system: Toluene: Ethyl acetate: Glacial acetic acid (7:2:1) (a) Visible light (b) UV light (c) Iodine chamber

Table 3: Results of qualitative phytochemical analysis of various *P. indica* extracts

Chemical constituent	*Pet. ether extract*	*Chloroform*	*Ethanol*	*Acetone*	*Methanol*	*Hydroalcoholic*
Carbohydrates	-	-	-	+	+	+
Alkaloids	-	-	-	-	+	+
Terpenoids	+	+	+	+	-	-
Flavonoids	-	-	+	+	+	+
Tannins and phenolic compounds	-	-	+	+	+	+
Saponins	+	-	-	-	-	-
Protein and amino acids	-	-	-	-	-	-

Cold Maceration extraction method, + and – indicates the presence and absence of corresponding SMs in the test extract

Table 4: Results of total phenolic and total flavanoids contents

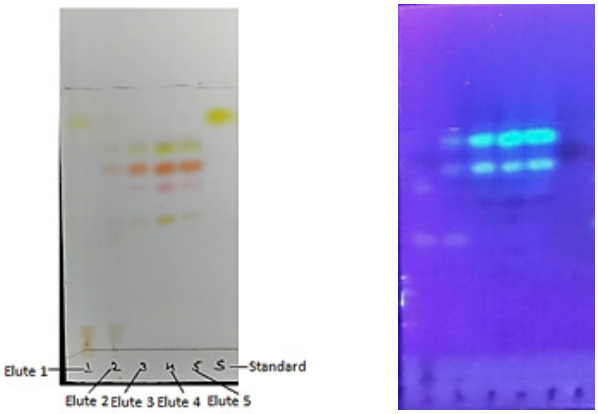
Extracts	*Total phenolic content (mg/gm equivalent to gallic acid)*	*Total flavonoids contents (mg/gm equivalent to rutin)*
Ethanolic extract	533. 83	132. 66
Hydroalcoholic extract	46. 667	219. 00

Table 5: Linear equations and R^2 for different standards used to calculate the results of different Assays

S. no.	*Name of assay*	*Name of standard and concentration*	*Linear equation*	R^2
1.	Total polyphenolic content (TPC)	Gallic acid (20–100 µg/mL)	Y = 0. 0025x + 0. 0823	0. 9856
2.	Total flavonoids content (TFC)	Rutin trihydrate (20–100 µg/mL)	Y = 0. 0014x + 0. 0823	0. 9437

Table 6: Results of antioxidant activity (DPPH) of different extracts obtained from the roots of *P. indica*

Extracts	*DPPH IC_{50} (%inhibition)*
Chloroform	65. 8
Ethanol	45. 47
Acetone	47. 26
Hydro-alcoholic	58. 64
Standard	27. 62



(a) (b)

Figure 5: TLC of elutes (a) in visible light (b) UV light

Determination of Extractive Value

To determine water and alcohol extractive values, about 5 gram of powdered drug (coarsely) was weighed into a 250 mL conical flask with stopper. To this 100 mL of distilled water and 100 mL of alcohol (99%) was added. During first 6 hours flask was placed on shaker. Then it was kept aside for 18 hours and then filtered. After that 25 mL of the plant filtrate was pipetted out and evaporated to dryness in a weighed shallow on When we

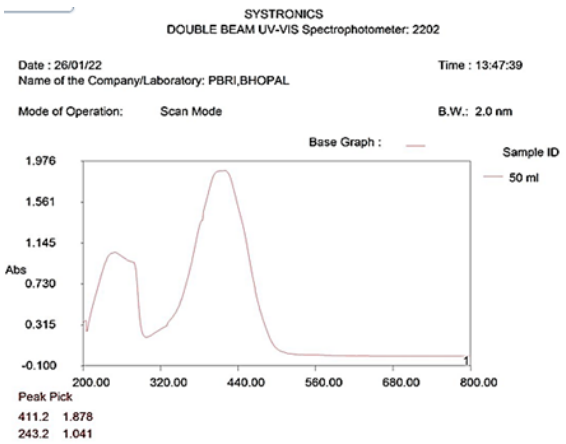


Figure 6: UV-visible spectra of elute 1

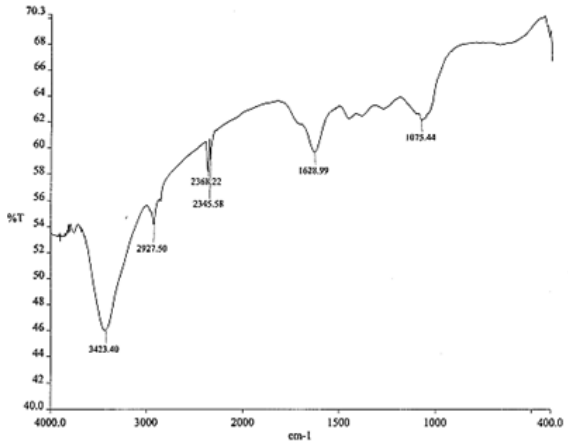


Figure 7: FT-IR spectra of elute 1

Table 7: Results of TLC of various extracts

Mobile phase - Toluene: Ethyl acetate: Glacial acetic acid (7:2:1)			
Extracts	*Rf value (in visible)*	*Rf value (in UV)*	*Rf value (in iodine chamber)*
Standard (S1)	0. 93	0. 93	0. 93
Acetone (S2)	0. 93, 0. 86, 0. 69	0. 93, 0. 79, 0. 71	0. 93, 0. 86, 0. 69, 0. 67
Chloroform (S3)	0. 93, 0. 86, 0. 69	0. 93, 0. 79, 0. 71	0. 93, 0. 86, 0. 69, 0. 67
Ethanol (S4)	0. 93, 0. 86, 0. 69	0. 93, 0. 79, 0. 71	0. 93, 0. 86, 0. 69, 0. 67
Hydro alcoholic (S5)	No spots	No spots	No spots
Methanol (S6)	No spots	0. 79, 0. 76, 0. 71	0. 67

Table 8: Results of TLC of elutes

Solvent system	*Elute*	*Visible light*		*UV light*	
		Spot	*Rf value*	*Spot*	*Rf value*
Toluene: Ethyl acetate: Formic acid (7: 3: 1)	Elute 1	2	0. 90	3	0. 90
			0. 43		0. 78
					0. 43
	Elute 2	4	0. 78	3	0. 94
			0. 70		0. 82
			0. 64		0. 43
			0. 43		
	Elute 3	4	0. 78	4	0. 94
			0. 70		0. 82
			0. 64		0. 70
			0. 50		0. 64
	Elute 4	4	0. 78	4	0. 94
			0. 70		0. 82
			0. 64		0. 70
			0. 50		0. 64
	Elute 5	4	0. 78	4	0. 94
			0. 70		0. 82
			0. 64		0. 70
			0. 50		0. 64
	Standard	1	0. 90	1	0. 90

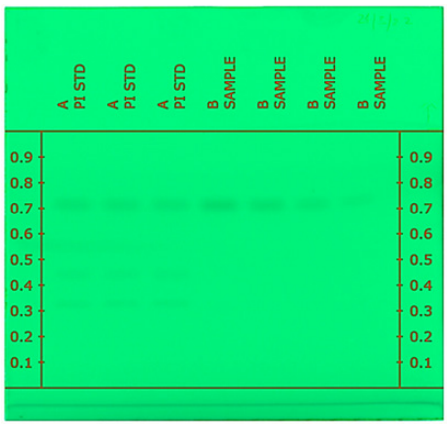


Figure 8: Chromatogram obtained from separation of plant extract and visualized under UV light of wavelength 254 nm.

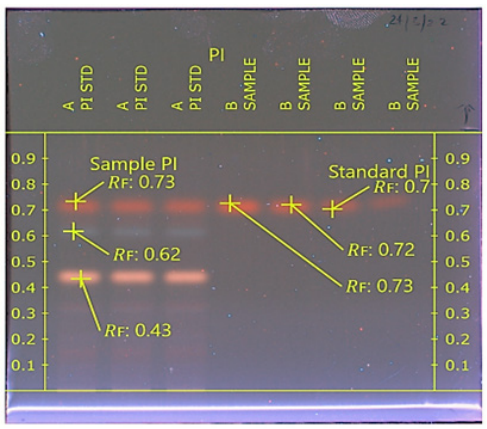


Figure 9: Chromatogram obtained from the separation of plant extract and visualized under UV light of wavelength 366 nm.

compared the Rf value of standard Plumbagin with all elutes, only elute 1 had the same band of Plumbagin. So, it confirmed that elute 1 contains Plumbagin. a water-bath. The residue was then dried to a constant weight at 105°C. Percentage of water and alcohol-soluble extractive was calculated.[12]

Qualitative Phytochemical Estimation of Extracts

Phytochemical screening was performed on the *P. indica* extracts of the roots (obtained in solvents such as Pet. Ether, Chloroform, Ethanol, Acetone, Methanol, Aqueous and hydroalcoholic) to determine the presence of phytochemicals such as carbohydrates, alkaloids, flavonoids, glycosides, proteins and amino acids, saponins, triterpenoids and steroids, tannins and other phenolic compounds (Table 3). These seven plant extracts were obtained using Cold Maceration extraction techniques. To identify the constituents in the seven different plant extracts, specific qualitative phytochemical tests were performed.[9]

Quantitative Phytochemical Estimation

Total Phenolic Contents

The Folin-Ciocalteu reagent and gallic acid as standard utilized to quantify the TPC of different extracts by using standard method with a few changes. The results of the experiment were

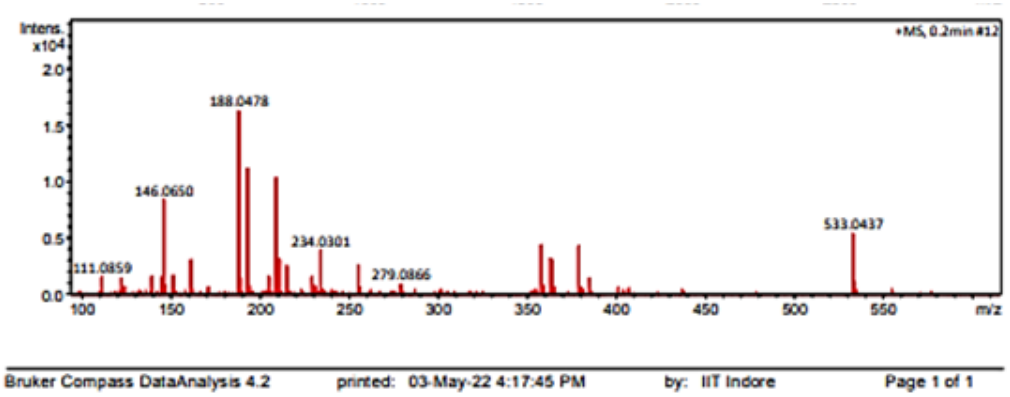


Figure 10: Mass spectra of Elute 1

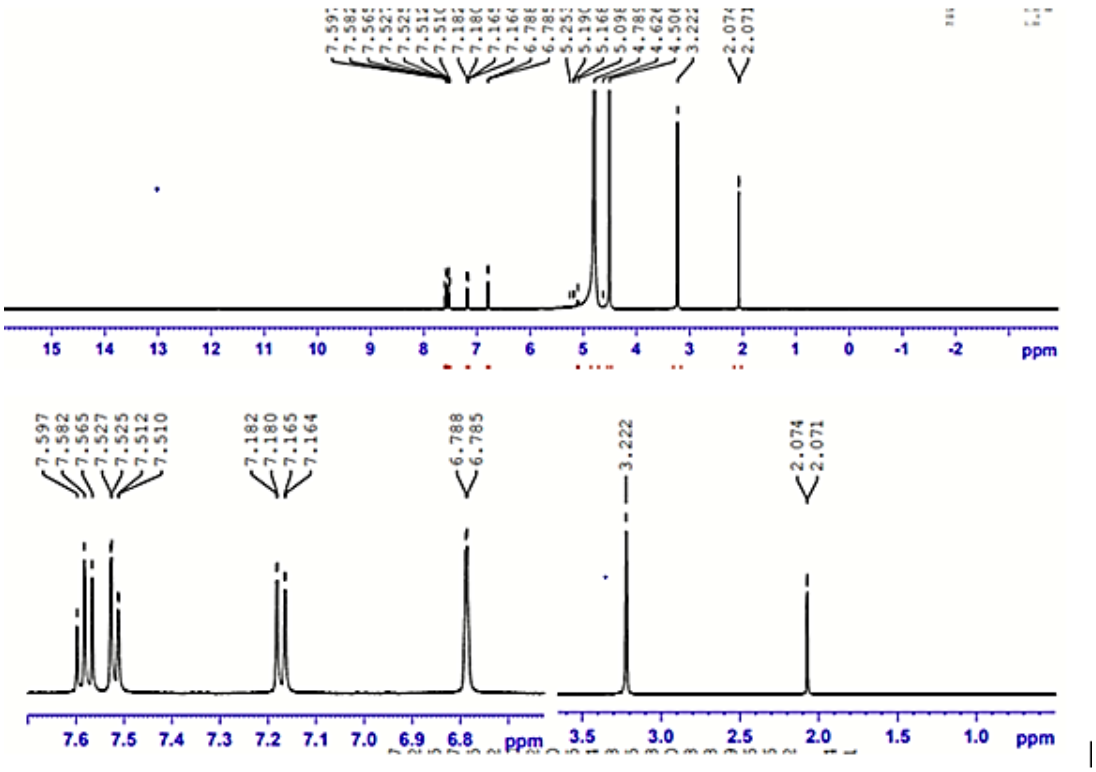


Figure 11: NMR spectra of Elute 1

calculated as mg of GAE (gallic acid equivalents) per gram of DPE (dehydrated plant extract). Different concentrations of Gallic acid (20–100 g/mL) in methanol and a test sample in methanol or a solvent with a similar polarity (0. 1 mg/mL or 100 g/mL) were prepared.

Different concentrations of Gallic acid (20–100 µg/mL) in methanol and test sample in methanol or a solvent of near about same polarity (0.1 or 100 µg/mL) were prepared. Then, 0.5 mL of different Gallic acid/test sample concentrations were mixed with 2 mL Folin-Ciocalteu Reagent (1:10 in deionized water). After allowing the mixture to stand at room temperature for 5 minutes, a 4 mL solution of sodium carbonate was added (7.5%). After thirty minutes of room temperature incubation with intermittent shaking, the sample absorbance (765 nm) were analyzed using methanol as blank, a UV-visible spectrophotometer was used and the Standard curve of Gallic acid with different concentration were plotted (Figure 3). TPC was determined and shown as mg/gm or µg/mg gallic acid equivalent (Table 4).[13]

Total Flavonoid Content (TFC) Estimation

The total flavonoid content of both extracts was determined using rutin trihydrate (standard) and aluminum chloride. This method measured TFC in milligrams of RE (rutin trihydrate equivalents) per gram of DPE (dehydrated plant extract). Various Rutin's concentration (20 to 100 µg/mL) in methanol and test sample in methanol or, a solvent of near about same polarity (100 µg/mL) was prepared. The deionised water

(2 mL) added in extract and standard (0. 5 mL) followed by 0. 15 mL solution of $NaNO_2$ (5% w/v). After 6 minutes incubation, 2 mL sodium hydroxide (4% w/v) and 0.15 mL $AlCl_3$ (10%, w/v) were mixed in whole solution. In a 5 mL volume, deionized water was added to the mixture solution and the mixture solution was left to stand for another 15 minutes. Finally, absorbance at 510 nm was measured by using UV spectrophotometer. The TFC of various samples was recorded v/s prepared water blank and articulated as mg/gm or µg/mg of rutin trihydrate equivalents (RE)/g by DPE. (Table 4) (Figure 3)[14] determined spectrophotometrically in terms of rutin equivalent, varied from 11.7 to 26.6 mgg^{-1} in spring leaves and 9.84 to 29.6 mgg^{-1} in autumn leaves. Fresh leaves gave more extract than air-dried or oven-dried ones. HPLC showed that mulberry leaves contain at least four flavonoids, two of which are rutin and quercetin. The percentage superoxide ion scavenged by extracts of mulberry leaves, mulberry tender leaves, mulberry branches and mulberry bark were 46.5, 55.5, 67.5 and 85.5%, respectively, at a concentration of 5 µg mL^{-1}. The scavenging effects of most mulberry extracts were greater than those of rutin (52.0%).

Antioxidant Potential Estimation

Stable DPPH Free Radical Scavenging Activity

2,2-Diphenyl-1-picryl-hydrazyl-hydrate free radical method was developed.[15] For the preparation of DPPH reagent, 0.1 mM solution of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) in methanol was prepared. A freshly prepared 1-mg/mL methanol solution of Ascorbic acid was then used. (Standard) 1-mg of *P. indica*/ standard test sample (extracts) was mixed with methanol to make a 1-mg/mL stock solution. Various volumes of extract/ standard (20–100l) were extracted from stock solution and placed in a set of test tubes and methanol was added to make the volume upto 1-mL. To this, DPPH reagent (2 mL of 0.1 mM) was added and mixed thoroughly. After that, absorbance was recorded at 517 nm after 30 minutes' incubation in dark at room temperature. For the control preparation, methanol (1-mL) was mixed with 2 mL of 0.1 mM DPPH solution and incubated for 30 min at room temperature in dark condition. Absorbance (control) was taken against methanol (as blank) at 517 nm.[15]

Percentage antioxidant activity of extract/standard was calculated by using the formula:

% Inhibition = [(Ab of control- Ab of sample/ Ab of control x 100]

Analytical Techniques

Thin Layer Chromatography

Thin-layer chromatography was carried out with pre-coated silica gel thin layer chromatogram sheet (Merck) as the stationary phase. The chromatogram is developed with a mixture of Toluene: Ethyl acetate: Formic acid (7:3:1) and dried at room temperature. The spots were visualized in Visible light, UV light and iodine chamber and Rf values were calculated.[16]

Flash Chromatography

Solvent system was optimized using TLC. BUCHI Flash Chromatography-MPLC Cartridges 12g Silica 40–63 µm was used. The Pump Manager C-615 controls the flow rate and monitors the current pressure of the pump modules 1 and 2. The maximum pressure for Pump Module C-615 is 10 bar (145 psi). 50 g of sample was mixed with solvent (ethanol), a total volume of 10 mL and column was saturated with solvent system (toluene: ethyl acetate: formic acid (7:3:1). The sample solution was injected into the sample injector and passed through MPLC Cartridges at a flow rate of 0. 5 mL/minute. 5. 0–5.0 mL fractions were collected.[17]

Ultraviolet-visible (UV-vis) Spectroscopy

Elute 1 was subjected to UV–vis spectroscopy at wavelengths of 200–800 nm to detect intense absorption peak.[18]

FT-IR Analysis

The functional groups of dried powders of various plant extracts were identified using a Fourier Transform Infrared Spectrophotometer (FT-IR). The translucent sample discs were made by combining 10 mg KBr salt and 1mg fine dried powder of various plant extracts. These powdered mixture pellets were packed in a Perkin Elmer FT-IR spectroscope (4 cm^{-1} resolution) with a frequency range of 4000 to 400 cm^{-1}.[19]

HPTLC Analysis

HPTLC was performed on silica gel 60 F_{254} 100×100 mm plate (Merk) with toluene: Ethylacetate: formic acid (7:3:1) as mobile phase. The standard (Plumbagin) solution (2 µL) were applied to the plate as 8 mm bands, application of the sample was performed with CAMAG-Linomat 5 Automated spray on band applicator equipped with 100 µL syringe and operated with the settings: band length 8 mm, application rate 150 nL/sec, application volume 0.2–0.8 µL for sample and 1.0 µL for standard, distance between track 14.4 mm, distance from the plate side edge 15.0 mm and solvent front position 70 mm. CAMAG TLC visualize 2 was used densitometrically to scan the bands. The scanner operating parameters were set to mode-absorption/reflection at an optimized wavelength of 254, 366 nm and in visible range.[20]

Mass Spectroscopy Analysis

Mass spectroscopy converts molecules into ions, which can then be separated and sorted based on their mass and charge. The mass spectrometer used for this purpose was a BeukerDaltonik Benchtop Easy-to-Use Electrospray Ionization. LC MS spectrometer with quadrupole time-of-flight.[21]

^{1}HNMR Spectroscopy

The isolated and purified compound's ^{1}H-nuclear magnetic resonance (NMR) spectrum was examined in methanol-d_4 using an NMR (AVNACE NEO500 Ascend BrukerBioSpin International AG, Switzerland), DPX-500 MHz, with tetramethylsilane as the internal standard.[22] A renowned traditional medicinal plant, is being exploited extensively for its roots

which are employed in the preparations of many important herbal products (*e.g.,* Dashmularisht, Chitrakadivati).

RESULTS AND DISCUSSION

Microscopic Evaluation

Root Microscopy

Transverse section of the thin root bark of *P. indica* showed the presence of periderm,cork and cortex, whereas the transverse section of the thick rootbark of *P. indica* revealed the presence of sclereids, cork, secondary xylem and medullary rays (Figure 1).

Powder Microscopy

Microscopic observation of *P. indica* root revealed the presence of bordered pitted vessel, calcium oxalate crystals, starch grain, and cork with resin cell. It also revealed the presence of cork with stone cell, epidermis with starch, medullary rays radially cut, pitted fibers and scattered parenchyma (Figure 2).

Percentage Yield

Seven solvents with increasing polarities were used to extract of *P. indica* roots. The %yield of crude extracts ranged from 0.112 to 4.558%, depending on the solvent. When the %yields of the materials with various solvents were compared, methanol extract gave the highest yield (4.558%), while pt. ether, chloroform, ethanol, acetone, methanol, aqueous, and hydroalcoholic extracts provided 0.112, 0.151, 2.192, 1.786, 0.943, and 1.374%, respectively (Table 1).

Pharmacognostical Evaluation

The determination of a crude drug's pharmacognostical properties is critical because it supports in the detection and quantification of mishandling, adulteration, and the establishing of correct standards. Various pharmacognostical factors were explored, including extractive values, ash values, and moisture content, and the results are provided (Table 2). The ash values of a crude drug provide information about the earthy matter or inorganic content of the drug, as well as any other contaminants present. The extractive values are mostly used to determine whether a drug is exhausted or contaminated.

Qualitative Analysis of Phytochemicals

The phytochemical composition of different *P. indica* root extracts was evaluated using qualitative tests. The presence of phytochemicals can be seen in the various extracts (Table 3).

Curves generated from the area under the peak in TPC assays were used to obtain linear equations and linear regression (R^2) of various concentrations of standards. When the secondary metabolites in different extracts were counted, it was discovered that methanol and hydroalcohalic extracts had the highest number of secondary metabolites when compared to other extracts. The number of SMs in various extracts of different solvents is listed below in the following order: methanol extraction >hydroalcoholic extraction >acetone extraction >ethanol extraction >chloroform extraction >pt. Ether. The majority of solvent extraction methods produce nearly identical results, and there is no significant difference in qualitative phytochemical analysis.

Quantative Analysis of Phytochemical

Total Phenolic Content

Total polyphenolic content of hydroalcoholic extracts and ethanolic extracts was calculated using the Folin-Ciocalteu reagent; the TPC yield of both extracts is 46. 667 and 533. 83 mg of GAE/g of DPE, respectively. Polyphenolic compounds are polar in nature, with the greatest solubility in polar solvents. The results in (Table 4) were derived from a linear equation.

$$Y= 0.\ 0025X +0.\ 0823$$

This linear equation was derived from the gallic acid standard curve (Figure 3),where Y is sample absorbance measured with a UV-visible spectrophotometer, X is the gallic acid concentration calculated from the calibration curve and listed in (Table 5). The total polyphenol content P (mg of GAE/g of DPE) was obtained from the value of X.

$$P= X \times V/N$$

Where: V= volume of extract: N =weight of plant extract in g.

Total Flavonoids Content (TFC)

The flavonoids content was quantified with the help of aluminium chloride method. The TFC yields of hydroalcoholic and ethanolic extracts were 132.66 and 219.00 mg of RE/g of DPE, respectively (Table 4). The mentioned results were obtained (using the same method as for total polyphenolic content) from the linear equation derived by the standard curve (Figure 3)(Table 5) of Rutin trihydrate at various concentrations.

Antioxidant Potential

DPPH Radical Scavenging Activity

The DPPH free radical scavenging activity was performed with different Plumbagin extracts in this method, and the percentage of inhibition ranged from 45.47 to 65.8%. The results showed that ethanolic extract obtained had the greatest free radical scavenging activity than all the other extracts (Table 6).

TPC and TFC had a strong correlation with DPPH. Thus, the higher value of free radical scavenging activity in polar solvent extracts compared to less polar solvents is attributed to polyphenolic and flavanoids content.

Analytical Techniques

Thin Layer Chromatography

TLC of different Plumbagin extracts was performed and results are summarize in (Table 7) (Figures 4 and 5).

Flash Chromatography

Flash chromatography of different Plumbagin extracts was performed. From this, five elute were collected and TLC of all the elute was performed (Table 8). Results revealed that elute 1 contained Plumbagin and examined further.

UV-vis Spectroscopy

Elute 1 contained Plumbagin, so UV- vis spectroscopy of elute 1 was performed and two peak were obtained at 411.22 nm and 243.2 nm (Figure 6).

FT-IR Spectroscopy

IR spectrum of elute was interpreted and the functional groups were identified. The first broad peak was found at 3423.40 cm^{-1} which confirmed the presence of phenolic alcohol group. Ketone (C=O) stretching peak was identified at 1628.99 cm^{-1}. Alkane (C-H) stretching and alkene (=C-H) bending peaks were found at 2927.50 and 1075.44 cm^{-1} (Figure 7).

HPTLC

The results from HPTLC chromatogram for *P. indica* extract were analyzed at 254 and 366 nm. The spots were identified at 366 nm. The extract evidenced 3 spots with R_f value of 0. 43, 0. 62 and 0.73. Spot 3 was identified as the presence of Plumbagin as compared with the R_f value of standard 0.7 (Figure 8 and 9).

Mass Spectroscopy

The molecular ion peak M+ of the compound (Plumbagin) was observed at m/e 188. 047 and is shown in the Figure 10.

NMR Spectroscopy

^{1}H–NMR spectra of Plumbagin was analyzed. The peak at 2.07 ppm in ^{1}H–NMR spectrum corresponds to the methyl group at 2nd position of the naphthalene ring. The protons attached at the 6 and 8 position in the naphthalene ring were featured at 7.18 and 7.16 ppm, respectively. The third carbon proton of the naphthalene ring appeared at 7.51 ppm. The peak at 7.58 was related to the seventh carbon proton. The peak at 3.22 was due to the residual peak of CD_3-OD used in NMR (Figure 11).

CONCLUSION

The cold maceration method was used to obtain extracts of the roots of the plant *P. indica*. The yield of compounds was higher in the polar solvent, methanol, and the probable reason may be a higher concentration of polar constituents. The pharmacognostic parameter was assessed. The total polyphenolic and TFC of ethanolic and hydroalcoholic extracts were determined. In general, more polar solvents had higher TPC and TFC values. The ethanolic extract has a higher antioxidant capacity as measured by free radical scavenging activity in DPPH. It might be explained by the high concentrations of TPC and TFC in the polar solvent.

The following conclusions have been listed:

- The polar solvents used for extraction in the techniques, *i.e.* cold maceration, had higher total polyphenolic and flavonoid contents and thus demonstrated good antioxidant activity than other extracts.
- The presence of *Plumbagin* and the presence of antioxidant activities due to the existence of bioactive compounds was demonstrated by TLC, FLASH, UV, FT-IR, and HPTLC analysis of various extracts.
- The plant *P. indica* has a high antioxidant capacity.
- Because of their antioxidant properties, various crude extracts of *P. indica* roots could be used in pharmaceutical and agricultural industries.
- The future scope of this work will include the formulation of *P. indica* preparations as an antioxidant agent and the isolation and derivatization of new noble bioactive compounds.

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