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Isolation and Characterization Plumbago Indica L. Root Extracts

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KEYWORDS
Chromatography, Plumbago indica L, Plumbagin, Phytochemicals, Spectroscopy

ABSTRACT:
The active ingredient of Plumbago indica L.,Plumbagin, has long been associated with a number of pharmacological actions; however, little is known about their toxicity. For the purpose to determine the crude drug's purity, it underwent pharmacognostic assessment and standardization. Later that the extract of plants underwent qualitative phytochemical analysis, and its total phenolic and flavonoid content was quantified. Extract isolation with thin layer Chromatography, Column Chromatography, UV-visible Spectroscopy, FT-IR, NMR, and Mass Spectroscopy.Following the extraction of Plumbago indica L. root powder, the methanolic, ethyl acetate, and chloroform extract contained the highest total flavonoid content, which was determined using Rutin as a standard. The total phenolic content of the extract was also measured in relation to Gallic acid confirming the primary functional group present in the sample by isolating and characterizing the extraction using Thin Layer Chromatography (TLC), Column Chromatography (CLC), UV-visible Spectroscopy (UV-VIS), FT-IR, NMR, and Mass Spectroscopy.The study project's outcomes indicated that Plumbago indica L., roots are rich in phytochemicals, and the presence of Plumbagin is the main source of Plumbago indica L significant several activities. The identity and purity of the compound was confirmed by Thin layer Chromatography, Column Chromatography, UV-visible Spectroscopy, FT-IR, NMR, Mass Spectroscopy. These data strongly support the possible utility of these extracts in disease prevention and treatment. Further the purpose of this study is to formulate (Herbosome) and investigate the effects of Plumbago indica L root extract and Plumbagin on hepatic malignancy.

INTRODUCTION

For Many years, people have turned to medicinal plants as a primary source of treatment for many human ailments[1]. In developing nations, the extraction and identification of pharmacologically active chemicals from medicinal plants has become more important. In order to clarify analytical markers, drug discovery approaches have been used more recently to standardize natural medicines[2]. The main benefits of medications made from plants are their effectiveness, affordability, and little or non-existent adverse effects.

In the Indian subcontinent, Plumbago species are a common plant species. Plants utilized in the traditional Indian medical system. One of the alkaloids found in P. indica roots is called Plumbagin (5-hydroxy-2methyl-1, 4-naphthoquinone), and it has a number of pharmacological properties, including antifungal, antibacterial, anti-parasitic, and antifertility properties and much more [3].

This study set out to examine and clarify the phenolic content of P. indica root extracts. The formulation of (Herbosome) and the investigation of Plumbago indica L root extract and Plumbagin effects on hepatic malignancy are additional goals of this study.

Material and Methods:

Collection of Plant Material:

The Plumbago indica L. (Family: Plumbaginaceae) plant that was used for this study was gathered from Maharashtra. Following collection, appropriate herbarium sheets were created for each plant, containing all of the fundamental data about that particular plant.

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These sheets were then sent, together with S. No. 149 dated 10.03.2022, and the specimen number, 149/Saif/Sci/Clg/Bpl, to Saifia Science College, Bhopal (affiliated to Barkatullah University, Bhopal, India). Following identification, they gave us the plant's scientific name.

Extraction:

Initially, undesired plant debris or plant pieces were removed from the gathered plant, especially the roots. At 35–40°C, the plants were then shed and dried.Using a suitable grinder, the thoroughly dried plants were reduced to a coarse powder. Until extraction started, the course powder was carefully preserved in an airtight container consisting of an inert substance that acts as though it does not react with powder. It was kept in a dry, cold, and dark environment. A thimble of soxhlet equipment was filled with 350 grams (350gms) of powdered Plumbago indica roots.Chloroform, ethyl acetate, and methanol were the three organic solvents used in the extraction process, which lasted eight to ten hours with the heating mantle temperature controlled between 40 and 60 degrees Celsius. Following the extraction procedure, the sample extract was filtered and dried out. Extracts were gathered and kept in an airtight container [4].

Formula of Percentage yield = $\frac{\text{Actual yield}}{\text{Theoretical yield}} * 100$

Pharmacognostic assessment

Plumbago indica L (Family: Plumbaginaceae) was the subject of a pharmacognostic study. Numerous methodologies are used in this work, such as an organoleptic examination and preliminary phytochemical research.

Organoleptic analysis

For research, Plumbago indica L's coarse powder was employed.

Total ash value:

A crucible made of silica, previously weighed and ignited, held around 5 g of powdered ingredients. A thin coating of the powder coated the bottom of the crucible. The powder was burnt by progressively increasing the temperature until it was dull red hot and carbon-free. After cooling, the crucible was weighed. The procedure was repeated to obtain a consistent weight. Using the air-dried powder, the percentage of total ash was determined [5].

% Ash content = $\frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Weight of crucible + sample - Weight of crucible}} * 100$

Loss on drying

Weigh a measuring vial that has previously been properly weighed after adding 2 to 6 g of the sample to it. After that, dry it for five to six hours at 105 degrees Celsius and chill it in desiccators loaded with silica gel. Once the material has dried to a fixed weight, the percentage of drying loss is computed [6].

LOD % = $\frac{\text{Wt. of petridish + crude drug - After drying}}{\text{Wt. of petridish + sample}} * 100$

Weight of crude drug

Water insoluble ash

For five minutes, the ash that was collected in accordance with the whole amount was heated in 25 milliliters of water. The insoluble material was collected and washed away with hot water over ash-free filter paper. After 15 minutes, the insoluble ash was placed in a silica crucible, ignited, and weighed. The procedure was repeated to obtain a consistent weight. The total weight of the ash was reduced by the weight of the insoluble particles. The difference in weight was thought to be water-soluble ash. Finding water-soluble ash content in proportion to the air-dried components [5].

% Water soluble ash = $\frac{\text{Weight of crucible + ash – Weight of crucible}}{\text{Crude drug weight}} * 100$

Acid insoluble ash

The ash was obtained as described above, and then it was heated for five minutes in 25ccof 2N Hydrochloric acid. The insoluble ash was collected and properly cleaned with hot water over ash-free filter paper. The insoluble ash was transferred into a crucible, heated, weighed, and then taken out. The procedure was repeated to obtain a consistent weight. Utilizing medicine that has been air-dried as a guide to calculate

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the proportion of acid-insoluble ash [5].

% Acid soluble ash =Weight of crucible + ash – Weight of crucible * 100
Crude drug weight

Alcoholic extractive value

Five grams of the powdered material were added to a 250 mL conical flask along with 100 mL of 90% ethanol and the stopper was replaced. After being mechanically shaken for six hours, the flask and its contents were allowed to stand for eighteen hours. An evaporating dish with a predetermined weight was filled with 20 mL of the filtrate from the mixture's filtering, and it was dried over an open flame. The steady weight of the residue was achieved after drying in the oven at 1050C for around three minutes [7].

The extractive value was calculated.
Water soluble extractive value = Weight of reside /Weight of the drug ×100

Water extractive value

Five grams of the powdered material were added to a conical flask fitted with a 250 mL stopper, 100 mL of water, and the stopper was then replaced. After being mechanically shaken for six hours, the flask and its contents were allowed to stand for eighteen hours. An evaporating dish with a predetermined weight was filled with 20 mL of the filtrate from the mixture's filtering, and it was dried over an open flame. The steady weight of the residue was achieved after drying in the oven at 1050C for around three minutes [7].

Water soluble extractive value = Weight of residue /Weight of the drug × 100

Plant extract quality phytochemical analysis

The Plumbago indica L root extracts were obtained and initially subjected to a preliminary phytochemical investigation by Kokate in 2006 [8]. The extract was examined for the presence or absence of numerous active components, including lipids or fixed oils, proteins, glycosides, phenolic compounds, alkaloids, flavonoids, saponins, amino acids, tannins, and carbohydrates.

Tests for Carbohydratesperformed byMolish Test,Fehling's Test, Benedict's test.
Tests for Alkaloids performed by the extract was mixed well with diluted hydrochloric acid before filtering. The filtrate was used for the subsequent experiments such as Mayer's Test, Hager's Test, Wagner's Test,Salkowski's Test, Libermann - Burchard's Test.
Tests for Flavonoidsperformed by Lead Acetate Test, Alkaline Reagent Test.
Tests for Tannin and Phenolic compoundsperformed by Ferric Chloride Test, Lead Acetate Test, Gelatin Test
Tests for Saponinsperformed by Froth Test
Tests for Fats and Oilsperformed by Solubility test
Tests for Protein and Amino acidsperformed by Biuret's Test, Ninhydrin Test.
Tests for Glycosidesperformed by Borntrager's Test, Legal's Test, Keller-Killiani Test

Quantitative Phytochemical estimation

Spectrophotometric Quantification of Total Phenolic Content

The total phenolic content of the Plumbago indica root extract was determined using the Folin-Ciocalteu Assay. The extracts were mixed with 2 mL of 7.5% sodium carbonate (0.2 mL from stock solution) and 2.5 mL of Folin-Ciocalteu Reagent. This mixture was diluted up to 7 mL with distilled water. Following two hours of room temperature storage for the final solutions, the absorbance at 760 nm was measured using spectrophotometry. Calibration curves were made using standard solutions of gallic acid equivalent (GAE) mg/gm. Gallic aid was prepared at concentrations of 20, 40, 60, 80, and 100 g/mL. One kind of reducing agent to which the Folin-Ciocalteu reagent is sensitive is polyphenol. They become blue when they react. This blue hue's spectrophotometric measurement [9].

Spectrophotometric Quantification of Total Flavonoid Content

The flavonoid content was established by use of the aluminum chloride procedure. A 0.5 ml solution of Plumbago indica root extract was mixed with 2 ml of purified water. Then, 0.15 milliliters of sodium nitrite (5%) was added and well combined. After that, add 0.15 ml of 10% aluminum chloride and let the solution sit for six minutes. Next, 2 cc of 4% sodium hydroxide was

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added. The liquid trembled and was well mixed. The absorbance at 510 nm of the combination was determined with a UV spectrophotometer. To construct calibration curves, rutin equivalent (GAE) mg/gm standard solutions were employed.

At doses of 20, 40, 60, 80, and 100 g/mL, rutin was synthesized. The total flavonoid content was determined using the calibration curve, and the results were expressed as milligrams of rutin equivalent per gramme of dry extract weight [10].

Isolation

Thin Layer Chromatography

On TLC plates of silica gel 60 F254 pre-coated with layer thickness of 0.2 mm, thin layer chromatography was performed using a solvent system consisting of toluene: methanol (9:1). The TLC chamber was produced at room temperature with the appropriate solvent solution, spots were manually placed using a capillary tube, plates were air dried using an air blower, and so on. Under UV, visible, and iodine light, spots on TLC plates were seen. The Rf value was computed [11].

Column chromatography

Plumbagin was isolated from Plumbago indica root extract using silica gel column chromatography and chloroform extract. Chromatography was performed using a vertical glass column constructed of borosilicate material. Before packaging, the column was thoroughly dried after being cleaned with acetone. Silica gel (# 60-120) was used as the adsorbent during the wet packing procedure used to load the column. Toluene was used to make the slurry, which was then poured into the column. A little amount of silica gel was placed to the column's top after combining 100 mg of extract in a sticky condition. The technique used for column chromatography was gradient elusion. Toluene was used to elute the column, and 4 eluents, each containing 10 ml, were recovered.

To determine the existence of a single component, TLC was used to concentrate the fractions recovered. Following that, 4 fractions were collected after the column had been eluted with toluene : methanol in a 9:1 ratio. TLC was used, and fractions were concentrated [12].

UV-visible Spectroscopy

Using the same solvent, the sample's separated fraction F3 was diluted to a ratio of 1:10. With the use of a UV-Visible Spectrophotometer (Shimadzu UV-1700), the extract was scanned between 200 and 800 nm in wavelength, and the distinctive peaks were found and noted.

FT-IR

FT-IR spectroscopy was carried out using a Perkin Spectrum BX spectrophotometer to determine if the functional groups were present in the isolated fraction (F3). Thermo Nicolet model 6700 spectrum spectrometer was used to analyze the materials after they were dried and crushed using KBr pellets. An IR spectrometer was used to evaluate a disk containing 50 mg of KBr that had been manufactured using a combination of 2% finely dried sample. The range of the infrared spectra that were acquired was 500–4,500 cm-1 [13].

NMR Spectroscopy

To determine the structure of the chemical included in the isolated fraction (F3), NMR spectroscopy was used. Fourier Transform Nuclear Magnetic Resonance spectroscopy, Model AVNACENEO500 Ascend BrukerBioSpin International AG, Switzerland, was used for this purpose [14].

3.6 Mass Spectroscopy

Molecules are transformed into ions by mass spectrometry, and the ions may then be sorted and segregated based on their mass and charge. The compound's molecular weight was determined using a mass spectrometer made by BrukerDaltonik, a high-performance, benchtop electrospray ionization system. Time-of-flight quadrupole LC MS spectrometer [14].

Results and Discussion

Thin Layer Chromatography

Upkeep for the Plumbago indica L. Methanol, ethyl acetate, and chloroform were extracted using several solvent systems (the solvent system was chosen based on a survey of the literature). Upon doing TLC at a 9:1 Toluene: Methanol ratio, the highest number of discernible spots were observed.

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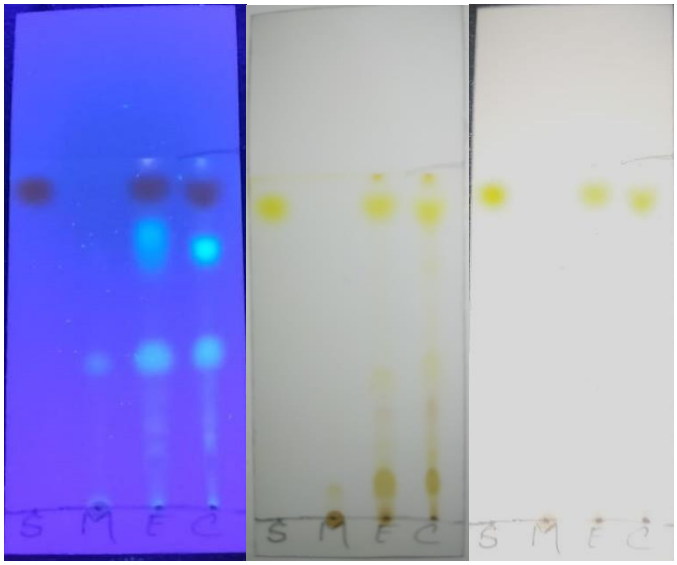
Table 1.TLC of Plumbago indica L.root extract

S. No.	Standard/Extract	Number of spots	R_f value	Solvent system
1	Methanol Extract	Spot 1	0.30	Toluene: Methanol (9:1)
2	Chloroform extract	Spot 2	0.09	
		Spot 1	0.95	
3	Ethyl Acetate extract	Spot 2	0.86	
		Spot 3	0.72	
		Spot 4	0.32	
		Spot 5	0.11	
		Spot 1	0.95	
4	Methanol Extract	Spot 2	0.86	
		Spot 3	0.69	
		Spot 4	0.32	
		Spot 5	0.11	
		Spot 1	0.41	
5	Chloroform extract	Spot 1	0.96	Hexane: Ethyl acetate (7:3)
6	Ethyl Acetate extract	Spot 2	0.92	
		Spot 1	0.99	
7	Methanol Extract	Spot 2	0.93	
		Spot 3	0.83	
		Spot 0	-	
	Chloroform extract	Spot 1	0.93	Chloroform: Methanol (6:4)
8	Ethyl Acetate extract	Spot 2	0.91	
		Spot 1	0.95	
9	Ethyl Acetate extract	Spot 2	0.88	
		Spot 3	0.75	

Figure 1. TLC of standard Plumbagin

a) UV light b) Iodine chamber c) visible light.



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Column Chromatography

Using TLC, the fractions/elutes from the Plumbago indica L. chloroform extract silica gel column chromatography were examined for the presence of several phytocompounds. One fraction included all of the phytocompounds with the same Rf values. Fractions isolated and finalized with different solvents using column chromatography in below table 2 and finalised fraction in solvent found Toluene: Methanol (9:1)

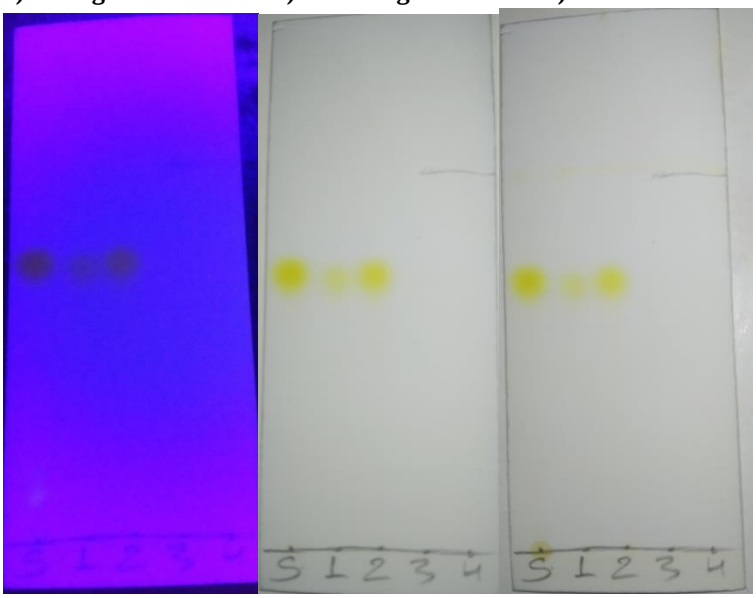
Table 2. TLC of finalised fractions

S. No.	Fractions	spots	R_f value	Solvent system
1	Fraction 1	No spot	-	Toluene: Methanol (9:1)
2	Fraction 2	1	0.95	
3	Fraction 3	1	0.86	
4	Fraction 4	1	0.72	
5	Fraction 5	1	0.72	
6	Fraction 6	1	-	
7	Fraction 7	1	-	
8	Fraction 8	1	-	
9	Fraction 9	2	0.32	
10	Fraction 10	2	0.32	
11	Fraction 11	3	0.32, 0.11	
12	Fraction 12	3	0.32, 0.11	

TLC of the isolated components was performed and the R_f value was compared with the standards. F3 fraction was found to show the same R_f with one of the standard (Plumbagin).

Figure 2. TLC of Fraction 3

a) UV light **c) visible light** **b) Iodine chamber**



UV-Visible Spectroscopy

The extract's separated fraction (F3) yielded UV spectra, which are displayed in Figures 3 and 4. The 200–800 nm range is where the peak was seen. 408 nm, 266 nm, and 214 nm were determined to have the greatest absorption.

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Figure 3. UV-Spectrum of isolated fraction (F3)

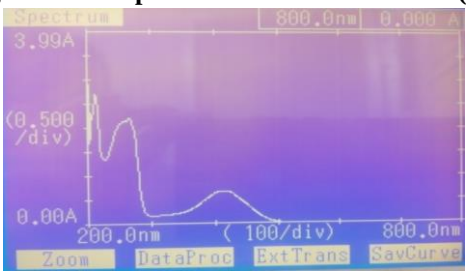
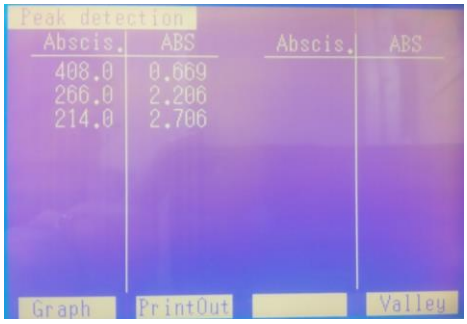


Figure 4.Absorbance of the isolated fraction (F3)



FT-IR

The functional groups were determined by interpreting the isolated fraction (F3)'s IR spectra. The major alcohol (-OH) peak was located between 3550 and 3200 cm-1, at 3432.23 cm-1. The alcohol (C-O) stretching peak was determined to be at 1385.35 cm-1, which is within the 1320–1000 standard range. The carbonyl stretching peak (C=O) was detected at 1627.33, falling within the normal range of 1760–1665. The bending peak of alkane (C-H) was determined to be 1059.53 cm-1. It attests to the presence of the sample's primary functional groups, ester, carbonyl, and alcohol.

Figure 5 FTIR of isolated fraction (F3)

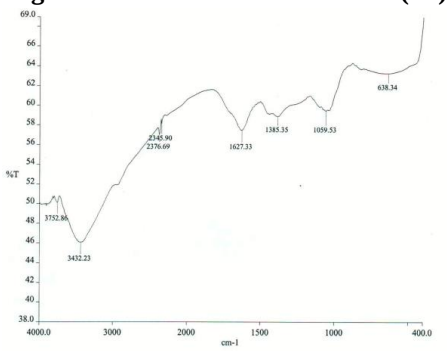
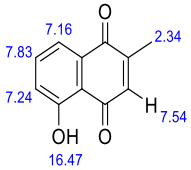


Table 3 FT-IR Interpretation

S. No.	Peak (cm^{-1})	Reference Range (cm^{-1})	Functional group present	Name of functional group
1	3432.23	3550 – 3200	O-H stretching	Alcohol group
3	1627.33	1760–1665	C=O stretch	carbonyl
4	1385.35	1320–1000	C–O stretch	Alcohol
5	1059.53	1000–650	=C–H bend	Alkane
6	638.35	900–675	C–H “oop”	Aromatic

NMRSpectroscopy

The isolated fraction's 1H-NMR spectra (F3) are displayed in Figure. In the 1H-NMR spectrum, the methyl group at the second position of the naphthalene ring is represented by the peak at 2.07 ppm. The naphthalene ring's protons at positions six and eight were present at 7.35 and 6.94 ppm, respectively. At 7.83 ppm, the naphthalene ring's third carbon proton was visible. The sixth carbon proton was associated with the peak at 8.03.



5-hydroxy-2-methylnaphthalene-1,4-dione

Figure 6 NMR of isolated fraction (F3)

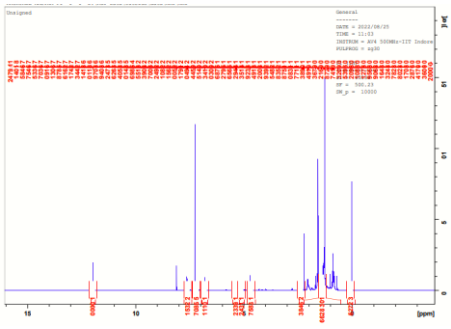


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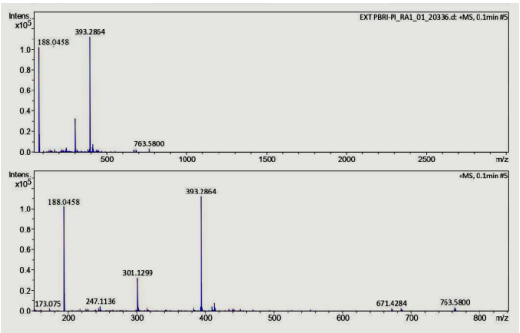
Table 4 Interpretation of NMR

S. No.	Obtained peak (ppm)	Predicted peak (ppm)	Group Present
1	2.07	2.34	Peak of methyl protons present at second position of naphthalene ring
2	3.22	3.31	Solvent residual peak of CD_3-OD
2	6.94,7.35	7.16, 7.24	Peak of the protons present at 6 and 8 position of naphthalene ring
3	7.83	7.54	Peak of the proton present at 3 position o f naphthalene ring
4	8.03	7.83	Peak of the proton present at 7 position of naphthalene ring

Mass Spectrometry

Observed at m/e 188.0458, the isolated fraction (F3)'s molecular ion peak M+ is broken up into many substructures. The substructures' molecular peaks were located at 173.075, 247.1136, 301.1299, and 393.2864. The Plumbagin peak and the compound's molecular ion peak are in agreement. The plumbagin chemical formula is $C_{11}H_8O_3$, and its molecular weight is 188.179.

Figure 7 Mass Spectra of isolated fraction (F3)



CONCLUSION

Currently, a sizable fraction of the world's plant population remains undiscovered, and it would seem plausible to argue that they may be the source of novel, safer, and more effective medications. The components of Plumbagin, which were isolated and characterized from the branches of P. indica root extract, have been presented in this work. It is well knowledge that medicinal plants have high antioxidant content and may be utilized to treat a wide range of illnesses. Our results demonstrate that the pharmacological effects of P. indica extracts may be ascribed to the presence of steroids, alkaloids, flavonoids, tannins, glycosides, and phenols in the crude extract. The potential usefulness of extracts in the prevention and treatment of illness is clearly supported by these studies. The goal of the study was to investigate and elucidate P. indica root extracts' phenolic content. Additional objectives of this study include the development of (Herbosome), the examination of Plumbago indica L root extract, and Plumbagin effects on hepatic malignancy.

Conflict of interest

The authors declare that there is no conflict of interest.

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